DESIGN, SYNTHESIS AND EVALUATION OF EXCESSIVELY CHARGED NUCLEOPHILES AS POTENTIAL REACTIVATORS OF AGED PHOSPHORYLATED ACETYLCHOLINESTERASES, AND N-BROMO IMIDES AS UNIVERSAL DECONTAMINANTS

By

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To Father and Mother

Good reasons must, of force, give place to better.

- Shakespeare

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KEY TO ABBREVIATIONS

CMC:	critical micelle concentration
CTAB:	Hexadecyltrimethylammonium bromide (co-surfactant)
DOD:	n-dodecyl group
But:	n-butyl group
Et:	ethyl group
PNPP:	p-nitrophenylphosphate moiety
PNP Phospn:	p-nitrophenylphosphonate moiety
2,4-DNPP:	2,4-dinitrophenylphosphate moiety
HA:	imidazolium hydroxamic acid
BzHA:	benzyl protected imidazolium hydroxamic acid
r.t:	room temperature
$t_{\frac{1}{2}}$:	half life, based on log of activity vs time
K^{1}_{2}:	apparent bimolecular rate constant
K_{obs}:	pseudo first-order rate constant
A_{∞}:	end point absorption
A_{t}:	absorption at time t
r:	correlation coefficient
accn:	reaction rate acceleration based on buffer hydrolysis
Exct:	excitation
Emiss:	emission
λ:	wavelength
log:	$\log_{10}$

M:	mole per liter
Bis:	disubstituted
mono:	monosubstituted
min:	minutes
sec:	seconds
nm:	nanometer
UV:	ultraviolet
μl:	microliter
ECNR:	excessively charged nucleophilic reagent

Abstract of Dissertation Presented to the Graduate School
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DESIGN, SYNTHESIS AND EVALUATION OF EXCESSIVELY CHARGED NUCLEOPHILES AS POTENTIAL REACTIVATORS OF AGED PHOSPHORYLATED ACETYLCHOLINESTERASES, AND N-BROMO IMIDES AS UNIVERSAL DECONTAMINANTS

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December 1989

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Major Department: Chemistry

Acetylcholinesterase (AChE) enzyme has a major physiological function in humans in ensuring that neurotransmission is of finite duration by cleaving neurotransmitter acetylcholine at the nerve junctions. The organophosphorus nerve agents inhibit this process by irreversibly phosphonylating or phosphorylating the enzyme, which results in elevated levels of acetylcholine, causing convulsion, muscular paralysis and death.

Inhibited AChE can be reactivated by conventional nucleophilic antidotes such as 2-PAM and Obidoxime. However, the inhibited enzyme "ages" from a reactivatable phosphate triester state to a nonreactivatable phosphate diester anion or an analog thereof, which is extremely inactive towards any nucleophiles due to anionic repulsion. So far, no antidotes have been shown to reactivate the aged enzyme.

A series of multicationic nucleophiles, namely, excessively charged nucleophilic reagents (ECNR), some having surface activity, have been designed, synthesized and investigated for the hypothesized mode of action: (a) neutralization of repulsive charge and (b) simultaneous nucleophilic attack against the aged enzyme models such as phosphate diester and phosphonate monoanions.

The ECNR carrying hydroxamate anion showed hydrolytic cleavage of the monoanions with 40-to 2,600-fold rate enhancement at pH 9.5. The reaction appears not to be influenced by micellar action but takes place by the hypothesized mode. The ECNR carrying oximate anions were less effective against the aged enzyme models. Both types of ECNR showed rapid acceleration of hydrolysis of organophosphate triesters. Some of the ECNR should have in vivo applications.

Decontamination by hydrolytic cleavage using positive halogen sources is one effective method to decontaminate nerve gas and mustard agents in field or under various environmental conditions. Some stable, water soluble, "soft" brominating N-bromo 2-oxazolidinone systems, as well as an N-bromo glycouril system with increased molar positive bromine, were designed, synthesized and investigated for use as universal decontaminants. The water-soluble 2-oxazolidinone system showed high stability and activity against both mustard and nerve gas (organophosphate) agent models. The glycouril system is exceptionally stable as a solid.

All synthetic strategies and methods to target molecules and the structural characterization of products and side products as well as physical-chemical studies and kinetic processes are discussed.

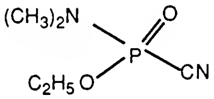
CHAPTER I
INTRODUCTION

This thesis will examine the design, synthetic methods, and evaluation strategies that are relevant to successful development of potential reactivators (antidotes) of organophosphate blocked "aged" acetylcholinesterases (AChE) and a new class of decontaminants of organophosphates.

Pharmacology of Acetylcholinesterase and Organophosphate Poisoning

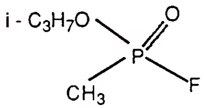
The organophosphate anticholinesterase agents (nerve agents) are highly toxic derivatives of phosphonic and phosphoric acid. Representatives of this class of compounds have widespread use in agriculture (e.g., parathion, malathion), pharmacology and medicine (e.g., DFP, paraoxon), and are of concern to the military because of their application as chemical weapons in war (e.g., tabun [GA], sarin [VD] and [VX]). The structures of these compounds and rat LD_{50} values are given in Figure 1-1.[1]

All of these organophosphates elicit their primary effects by phosphorylating or phosphonylating the serine hydroxyl group at the active site of the enzyme acetylcholinesterase, causing essentially irreversible inhibition of the enzyme. Differences in their ultimate pharmacological effects and their lethality (toxicity) are derived from their relative ability to penetrate into the central nervous system (CNS), their rate of reaction with AChE, and their selectivity for reaction with the enzyme at specific loci, and their behavior (e.g., aging) once attached at the active site on the enzyme.



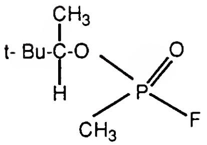
Tabun (GD)

LD_{50} 0.20



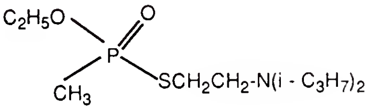
Sarin (GB)

0.14



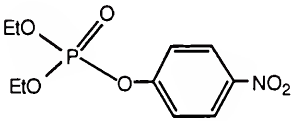
Soman (GD)

LD_{50} 0.10



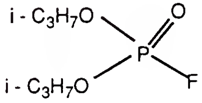
VX

0.02



Paraoxon

LD_{50} 0.50



DFP

3.00

LD_{50}(rat) mg/kg, Route sc

Figure 1-1: Organophosphorus anticholinesterase agents.

Normal Function and Inhibition of AChE

Acetylcholine is found as a metabolite in high concentrations in nervous tissue and in motor nerve tracts, as an active neurotransmitter substance. When a nerve cell enervates a muscle fibre, the nerve-cell ending at the muscle junction forms a morphologically distinct and functionally discrete contact called a synapse. This is a gap (typically of about 500 Å) between the presynaptic nerve-ending membrane and postsynaptic membrane of the muscle-cell membrane. This interaction transmits a depolarization signal to the muscle membrane in a manner as yet imperfectly understood.

The physiological role of acetylcholinesterase is to ensure the nerve impulse is of finite duration by lowering the acetylcholine concentration in the cleft via hydrolysis. This enzymatic cleavage will lower the concentration of free acetylcholine in the synaptic cleft, and the resulting perturbation of equilibrium will induce dissociation of the acetylcholine-acetylcholine receptor complex, turning off the signal to the muscle cell. Active acetylcholinesterase is crucial for normal neuromuscular function, and inhibition of this enzyme produces tetanic shock, with eventual muscle paralysis. In severe cases asphyxiation can result. Not surprisingly, this enzyme is a target for nerve agents and insecticides such as Soman and diisopropylphosphofluoridate (DFP).

AChE is loosely associated with postsynaptic membrane.[2] As a catalytic entity, AChE is remarkably efficient, with a turn over number (K_{cat}) of 25,000 sec^{-1} and will cleave a substrate molecule once every 40 μsec. The active site of AChE as depicted in Figure 1-2, is considered to consist basically of two subsites: an anionic site, possibly represented by a glutamate ion, and an esteratic site, located about 2.5 Å from the anionic site which has been shown to incorporate a serine moiety and may contain a histidine residue (pK_a 7.2) and a tyrosine residue (pK_a 9.3).[3] A hydrophobic area at the active site is also indicated.

In normal functioning, acetylcholine, after acting at a cholinergic receptor, complexes reversibly with the active site of the enzyme. In a fast step, the acetyl group is transferred from the acetylcholine molecule to a serine hydroxyl group, forming acetylated enzyme and releasing choline. This is followed by a rate limiting hydrolysis of the acetate ester group, presumably acid catalyzed by the imidazolium ion and possibly also aided by nucleophilic action of the tyrosine hydroxyl, releasing acetate anion and providing reactivated enzyme available to hydrolyze another molecule of acetylcholine. The released choline is transported back into the nerve ending for reconversion to acetylcholine and storage.

It is recognized that, although the active site has been fairly well elucidated, acetylcholinesterase is an enzyme of highly complex structure that has by no means been fully characterized. The enzyme appears normally to consist of several subunits, and enzyme obtained from different species and different tissues (e.g., erythrocyte vs. brain) can show significant quantitative differences in sensitivity to organophosphorus agents and ability to be reactivated after inhibition.

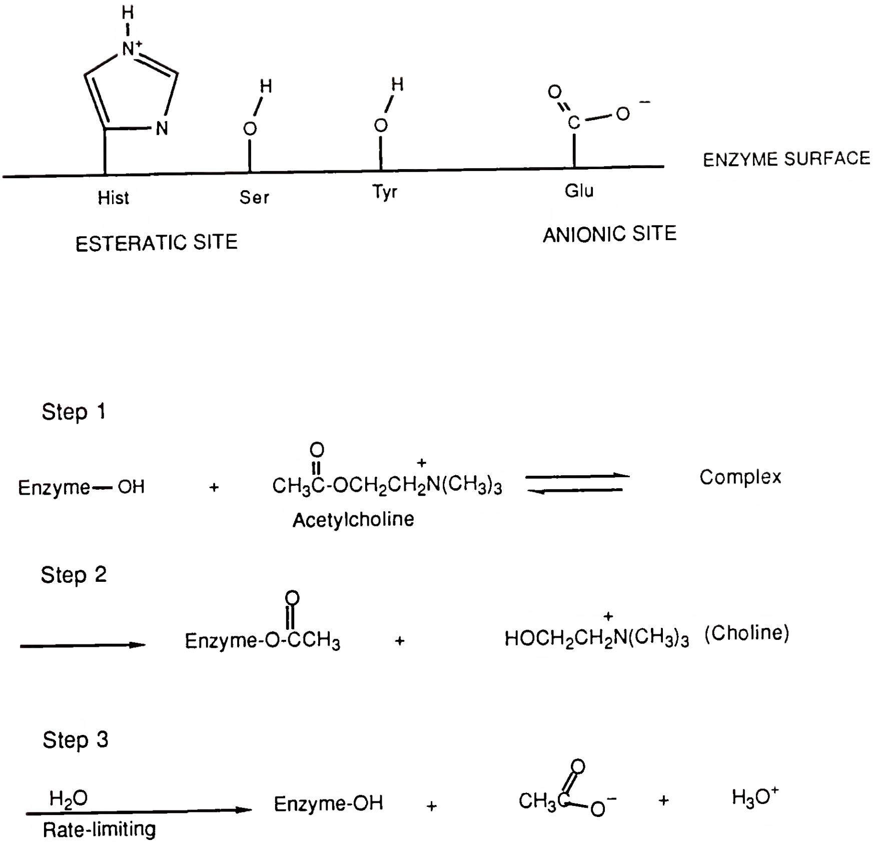

ENZYME SURFACE
Hist
Ser
Tyr
Glu
ESTERATIC SITE
ANIONIC SITE
Step 1
Enzyme— OH + CH3C-OCH2CH2N(CH3)3 ⇌ Complex
Acetylcholine
Step 2
→ Enzyme-O-CCH3 + HOCH2CH2N(CH3)3 (Choline)
Step 3
H2O
Rate-limiting
Enzyme-OH + CH3C—O⁻ + H3O⁺

Figure 1-2: Normal functioning of acetylcholinesterase.

The organophosphorus anticholinestrases react rapidly with the enzyme; the nerve agents react particularly rapidly. A lethal dose of Soman, for example, will have completely reacted within minutes of administration to an animal. A hypothetical formulation of the process is depicted in Figure 1-3. The more potent organophosphorus agents react so rapidly with enzyme (in seconds), initial kinetic studies indicated the reaction to be a simple bimolecular process without intervention of a binding complex of the reversible Michaelis-Menton type. Since the reaction is stoichiometric, one molecule of inhibitor abolishing the functioning of one enzyme active site, the extreme toxicity of the organophosphorus agents can be understood when one considers that acetylcholinesterase is present in the body in only small amounts.

The organophosphorus esters act by acylating the serine hydroxyl group at the active site of AChE, causing essentially irreversible inhibition of the enzyme with consequent elevation of acetylcholine levels. Acetylcholine accumulates in the peripheral and central nervous system (CNS). Ultimately, at sufficiently high level there is depression of the respiratory center in the brain abetted by peripheral neuromuscular blockage, causing respiratory paralysis and death. Generally more lipophilic, organophosphorus esters show high neurotoxicity as the result rapid penetration to the CNS system. However, the acute toxicity effects of the organophosphorus anticholinesterase agents are elicited by the elevated acetylcholine levels resulting from inhibition of the enzyme.

In sharp contrast to the rapid hydrolysis of the acetylated enzyme, hydrolysis of phosphorylated or phosphonylated enzyme is extremely slow, with a half-life typically of the order of hours to days.[3] Thus the enzyme is effectively inactivated and prevented from carrying out its normal function of hydrolyzing acetylcholine.

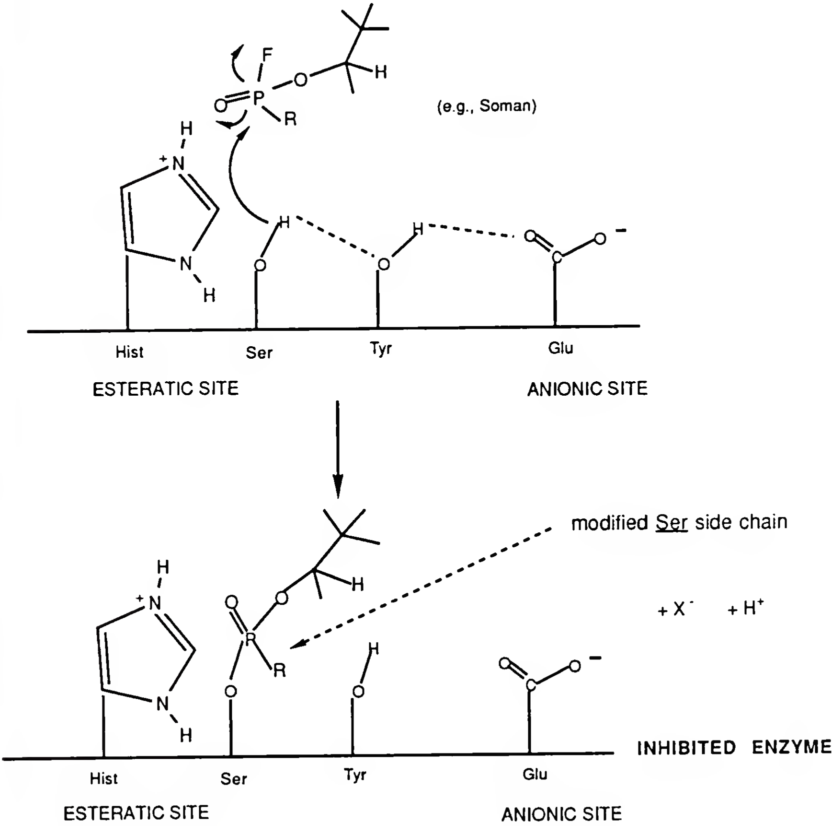


Figure. 1-3: Inhibition of acetylcholinesterase by an organophosphorus derivative.

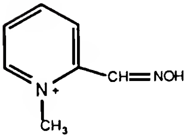
Reactivators (Antidotes) of AChE

Despite considerable effort taken in design of antidotes (particularly by the military ever since the development of Tabun in Germany in 1937) no fully satisfactory therapeutic or prophylactic agents have yet been devised.[2] One of the most effective reactivation nucleophiles yet found is pyridine-aldoxime methiodide, or 2-PAM. It illustrates rational drug design based on a knowledge of enzyme mechanism. The military has considerable interest in prophylactic as well as therapeutic agents, the former intended for protection of personnel in the event that nerve agent attack appears imminent.

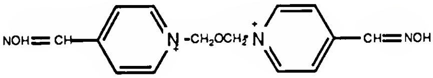
Design of antidotes has focused on essentially two ways to counter the toxicity of organophosphorus derivatives, namely (1) blocking the cholinergic effects of the elevated acetylcholine levels, and (2) reducing those levels. So far no single compound acting in either of these ways has proved completely satisfactory. Efforts to achieve reduction of the elevated levels of acetylcholine induced by the organophosphorus nerve agents have largely focused on the development of nucleophilic agents that can reactivate inhibited AChE by cleaving the bond attaching the phosphoryl group to the serine hydroxyl at the active site.

A large number of nucleophilic agents were prepared and tested in the early 1950s; two groups, hydroxamic acids and oximes, emerged as promising reactivators. With respect to hydroxamic acid, the effective nucleophile is hydroxamate anion, at physiological pH. However, the oximes appeared to offer more promise than hydroxamic acids. The prototype agent for this purpose is an oxime derivative, 2-

pyridinealdoxime methiodide (1, 2-PAM) or methyl methylsulfonate (P2S), designed some years ago by Wilson et al.[4] Since then structure-activity relationship among the huge number of analogues of 2-PAM that have been prepared have been studied, Obidoxime (2,Toxogonin) has been found to be somewhat more effective in reactivating AChE in animals.



2-PAM (1)



Obidoxime (2)

A hypothetical picture of the way in which 2-PAM may reactivate inhibited enzyme is presented in Figure 1-4. The pyridinium ion complexes with carboxylate anion at the anionic site, anchoring the oximino anion in position to displace the phosphorus ester group from the electrostatic site. The tyrosine and the histamine residues possibly assist in the displacement. It should be noted that reactivation with 2-PAM requires the anti-isomer of 2-PAM; the anti-syn interconversion is a very facile process that may occur in the oximate form at the enzyme level.[5]

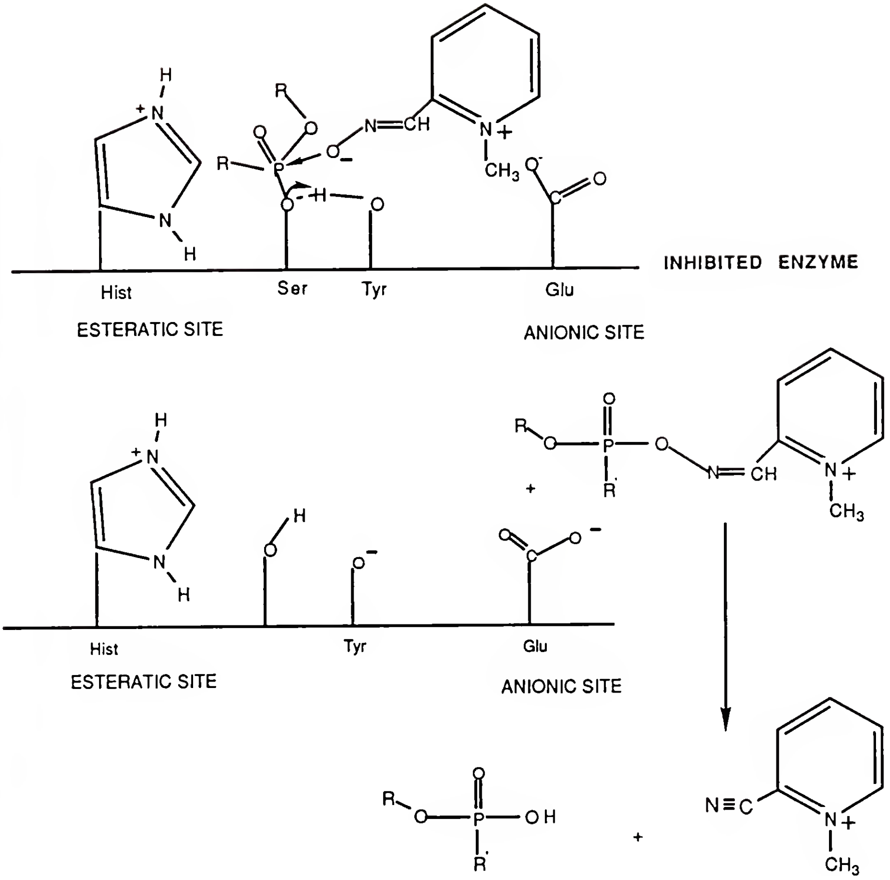


Figure 1-4: Reactivation of inhibited AChE by a conventional oxime (2-PAM).

2-PAM chloride, in conjunction with atropine, has generally been adopted in the United States for treatment of poisoning by organophosphorus esters, whereas Obidoxime is preferred in some European countries and the methasulfonate salt of 2-PAM (P2S) in others. However, a major drawback of all available nucleophilic reactivators is that they cannot reactivate aged enzyme.

Aging of Inhibited Acetylcholinesterase

It is generally accepted that after an organophosphate reacts with the AChE, a process of aging of the inhibited AChE will take place.[6] This aging is due to the transformation of the inhibited enzyme from a form which can be relatively easily reactivated by appropriate nucleophiles, to one which cannot be dephosphorylated at all. The time required for aging depends very much on the type of organophosphates involved. Thus AChE inhibited by DFP is transformed into the non-reactivatable form in several hours. In contrast, aging due to Soman occurs in 2 minutes (Table 1-1). The aging process of Soman inhibited AChE is depicted in Figure 5. It was suggested [7] that the rate determining step in the aging reaction of an alkylphosphorylated AChE is the fission of one of the C-O bonds. This theory was substantiated by Michael et al.[8] The loss of the pinacolyl group, probably by an acid-catalyzed process, and presence of the imidazolium ion may play critical role in initiating the process.[9] This aging process transforms the inhibited enzyme from a form which can be relatively easily reactivated by appropriate nucleophiles, to one which cannot be dephosphorylated at all.

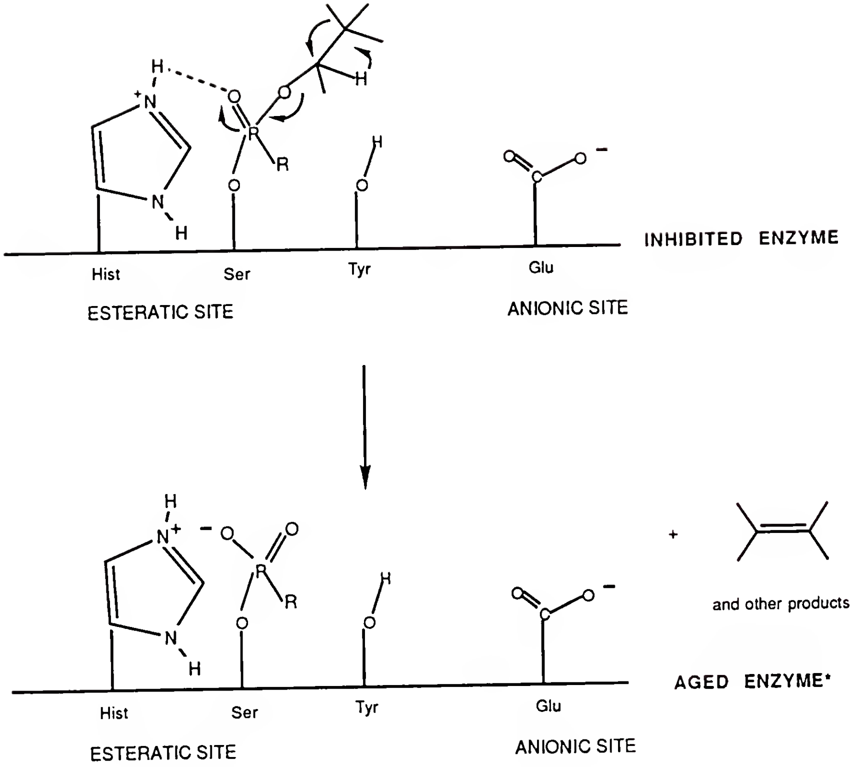


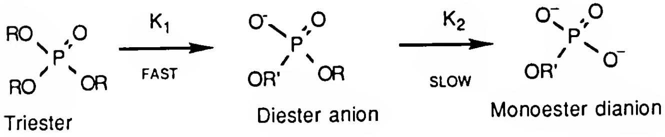
Figure 1-5: Aging of the poisoned (phosphorylated) AChE.

Chemically, it is evident that according to the aging process the main change is essentially the transformation of a neutral phosphate ester (neutral triester) into a negatively charged phosphate ester (diester anion). The diester anion is significantly more stable than the original neutral triester, for the following reasons: the first is an electrostatic effect, a direct repulsion between the diester anion and the attacking OH^- (hydrolysis) or other nucleophilic anion (acetate, phosphate, carbonate, oximate, hydroxamate, thiosulfate, etc.). The second factor is the decreased reactivity of the phosphorus because of the decrease in its partial positive change. As a consequence of these effects, the second-order rate constants for attack by anionic reagents (nucleophilic reagents) are from 2,000 to 7,000 times smaller for the diester anions than for the triester.[10]

Table 1-1: Half lives of aging of some organophosphate derivatives of acetylcholinesterase.[3]

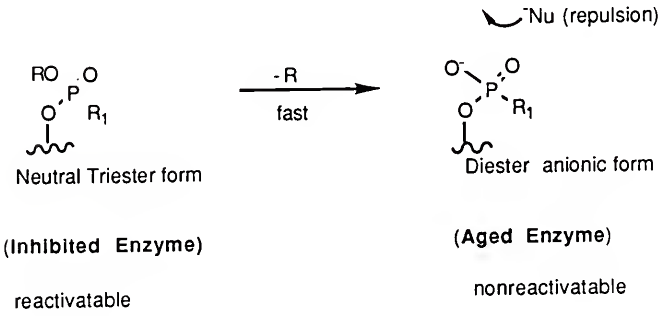
AChE-Nerve agent	$t_{1/2}$
DFP	4h
GA (Tabun)	14h
GB (Sarin)	3h
GD (Soman)	2-6 min

The anions of simple phosphate diesters are generally exceedingly unreactive.[11] Since the pKa of the dialkyl hydrogen phosphate is between 1 and 2, the species present under most physiological conditions after the aging takes place is the anion, and consequently the first problem to be solved is the acceleration of hydrolytic cleavage of phosphate diester anions.



Eqn 1-1

K_1 / K_2 = 2,000 - 7,200 times



Eqn 1-2

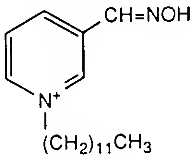
Various efforts have beeen made to circumvent the problem of reactivation of aged enzymes. Among these are (a) the design of agents capable of realkylating the enzyme bound phosphate anion, converting it back to neutral triester and thus making it again susceptible to nucleophilic reactivators such as 2-PAM (to date, no such alkylating agent has been found); (b) investigation of agents to slow the rate of aging in vitro (certain amines and ammonium salts, particularly bispyridinium salts, have been found capable of retarding the rate of aging in vitro)[12]; (c) investigation of prophylactic treatment of AChE temporarily with a reversible inhibitor, thus temporarily preventing a subsequent dose of organophosphate from gaining access to the physiologically important enzyme[13]; and (d) development of reactivators with the concept that a suitably constructed nucleophilic reagent might be capable of displacing the anionic phosphorus ester group.

This work is specifically aimed on approach (d) to develop conditions and specific new compounds which will solve one major problem related to organophosphate poisoning; effective reactivation of aged blocked AChE.

At physiological pH hydroxamic acid and oximes are largely ionized, and the anions are effective nucleophiles. In addition to the pK_a of the reactivators, other physicochemical properties of the reactivating agent, including steric and electronic factors and lipophilic - hydrophobic balance, can influence relative reactivating ability.

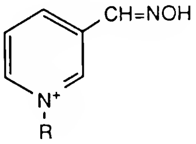
Cationic Nucleophiles

It was shown by several authors [14] that the active forms of the common nucleophilic reagents are the corresponding anions (hydroxamate, oximate, imidazole anion, thiolate, alkoxide, etc.) which have increased reactivity in cationic micelles. Within the past two decades, a number of micellar systems such as 5 - 10 have been investigated in which functional groups have been incorporated into micelle forming molecules. It was proved by Bodor et al.[15-17] that a molecular combination of anionic nucleophile and a cationic surfactant results in a highly effective accelerator of hydrolysis of various organophosphates. Thus, the micellar n-dodeceyl-3-(hydroxyiminomethyl)pyridinium salt (3) or the corresponding 1-n-heptyl (or nonyl or hexadecyl) compounds (4) were found to be much more effective nucleophilic reagents for the reaction with well known organophosphates such as Paraoxon (diethyl p-nitrophenyl phosphate) and O-ethyl S-2-diisopropylaminoethylmethyl phosphonothiolate (VX) than were nonmicellar analogs of 1-alkyl-3-(hydroxyimino methyl) pyridinium salts.



3

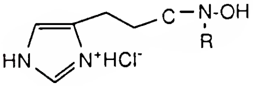
X= I (a)
= Cl (b)
= Br (c)
= CH_3SO_3 (d)
= CH_3PhSO_3 (e)



4

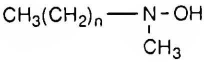
R = C_7H_{15} (a)
= C_9H_9 (b)
= $C_{16}H_{33}$ (c)

Epstein et al.[15] have shown that 3(a) brought the half life of the hydrolysis of paraoxon to 17.5 minutes from 10,500 minutes at slightly basic pH (9.3). They also showed that hydrolysis of VX can be significantly accelerated by 3. The various homologs of 3 such as 4(a-c) have also shown dramatic acceleration of hydrolysis of Paraoxon when used above their critical micelle concentration (CMC).



R = dodecyl

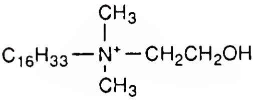
5



$CH_3(CH_2)_n-\underset{CH_3}{N}-OH$

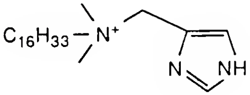
R= CH_3 or $CH_2N(CH_3)_2$

6

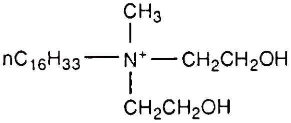


$C_{16}H_{33}-\overset{CH_3}{\underset{CH_3}{N^+}}-CH_2CH_2OH$

7

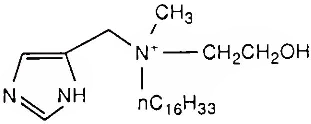


8



$nC_{16}H_{33}-\overset{CH_3}{\underset{CH_2CH_2OH}{N^+}}-CH_2CH_2OH$

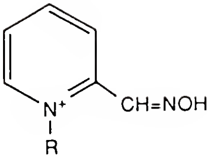
9



10

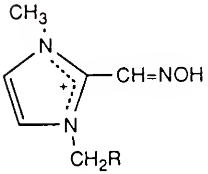
Kunitake et al.[18] introduced a chymotrypsin model bifunctional nucleophilic reagent containing an imidazole moiety substituted at the 4-position of the ring with an alkyl hydroxamic acid (5). In mildly basic conditions this reagent caused dramatic, enzyme like acceleration of hydrolysis of p-nitrophenyl acetate. It was also shown that imidazole containing quaternized choline type analogues catalyze phosphate hydrolysis more than does simple quaternized choline. Hydroxamic acid micellar nucleophiles such as 6 have shown good acceleration of hydrolysis of organophosphates at mild alkaline conditions (as specified for oximes).[19] Bodor and Kaminski[17] as well as Moss et al.[20] have found that N-hexadecyldimethylammonium ethanol bromide 7 accelerates micellar cleavage of organophosphates at basic pH (10.5 to 12). Bifunctional analogues such as 9 showed[17] reduced acceleration of hydrolysis of organophosphate compared to monofunctional 7. This is understandable if we consider that anions are the reactive species at alkaline pH; this would tend to retard reactivity due to repulsion of anionic species in the case of 9.

In vitro work[21] with a series of 2-PAM analogues at pH ~ 8.0, showed that the reactivity of the benzyl derivative (11(f)) is by far the highest, while the dodecyl derivative (11(e)) is most potent among the alkyl derivatives against Tabun inhibited AChE.



11

R = CH_3 (a)
= C_2H_5 (b)
= C_3H_7 (c)
= i-C_3H_7 (d)
= $C_{12}H_{25}$ (e)
= benzyl (f)

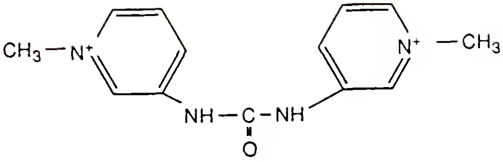


12

R = $O(CH_2)_nCH_3$ (a)
= OCH_2Pyridiniumaldoxime (b)
= $(CH_2)_nCH_3$ (c)
= unsaturated chain (d)

Very recently, Bedford et al.[22] have shown that some 3-substituted (hydroxyimino) imidazolium salts (12(a)) were effective in providing *in vivo* and *in vitro* reactivation of both Soman (GD) and ethyl p-nitrophenyl methyl phosphonate inhibited AChE (non-aged enzyme). Their findings suggest that the imidazolium ring system is a likely target for structural "fine tuning" to improve activity of poisoned AChE *in vivo*.

Pretreatment of enzymes with quaternary ammonium compounds including pyridinium oximes, has been shown[12] to have a retarding effect on enzyme aging due either to binding or to action on the acetylcholine (cholinergic) receptors. For example, compound 13 and its derivatives decrease aging of AChE by 4 fold.



13

Some quaternary amines which are allosteric effectors of AChE can retard aging (due to their binding points on the enzyme, distant from the active site), but this effect has no practical value as large concentrations of these effectors have to be used.[23] It is also found that imidazole and alkyl substituted imidazoles retard aging, presumably by binding to the anionic site on the enzyme. However, it might be possible that the undissociated form of the oximes accelerates the aging of the tabun inhibited AChE.

To date, no significant results have been reported for reactivation of aged AChE. The hydrolysis accelerating effect of quaternary ammonium compounds are marginal, a maximum 5-fold rate enhancement being achieved. Monoanion phosphonate and phosphates are about 5,000 times less susceptible to hydrolysis than are the triesters.

It is clear that the problem of reactivation of aged uninhibited AChE cannot be solved until conditions for acceleration of hydrolysis of the phosphate diester anions are found.

There are two things that an effective accelerator of phosphate diester monoanions must accomplish:

a. partial or complete neutralization of the negative charge, thus approaching the case of a phosphate triester hydrolysis; and

b. acceleration of the hydrolysis by an effective nucleophilic attack on the phosphorus.

Epstein et al.[24] postulated a "charge effect" theory, making uncharged pyridine aldoxime a very ineffective nucleophilic reagent for reactivation of inhibited AChE. It was also pointed out by Gray[1] that the electrostatic binding of the quaternary ammonium group to the enzyme anionic site would have the quaternary ammonium group carrying the nucleophile in a favorable position for reacting with the phosphorus ester groups thus facilitating reactivation.

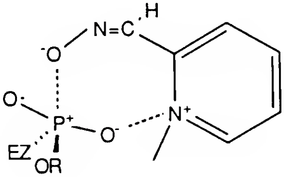


Figure 1-6: Diester anion -2-PAM complex.

A 2-PAM model may bind to the aged AChE model by a possible strong interaction between the positive charge of 2-PAM (anti) and the negative charge of the phosphate (Figure 1-6). However, no reaction is possible because of the reduced nucleophilicity of the oxime position due to the loss of charge effect exerted by the pyridinium positive charge.

One conclusion emerges from these observations: the need for "excessive positive charge" in the molecular combination for reactivating phosphate diester anions. An advantage presented by such a combination is that excessive positive charge should directly or indirectly facilitate the nucleophilic attack of the nucleophile (oximate, hydroxamate, etc.) without reducing its nucleophilicity. In solutions, such a combination effect was achieved[25,16,17] only in the case of neutral triesters, by mixtures of positively charged micelles and specific nucleophilic reagents. In vivo, however, a molecular combination of the "excessive positive charge" and effective nucleophile group, covalently bonded in the same molecule have to be used, primarily due to the differential transport problems typical for complex in vivo systems.

Specific Aims (Reactivators of Aged AChE)

This thesis suggests a novel and more flexible solution to the problem of reactivating aged inhibited AChE: the use of type nucleophilic reactivators having multiple positive charges for acceleration of the hydrolysis of the phosphate diester monoanion (or phosphonate monoanion). A systematic study of the reaction of properly designed "excessively charged nucleophilic reagents" (ECNR) carrying nucleophilic heads such as oximate or hydroxamate ions with simple phosphate diester anions and diester anions simulating an aged phosphorylated AChE will be used to evaluate the viability of this approach. The basic concept of this study is then to synthesize charged and multicharged functional nucleophilic reagents. For the in vitro modeling, some of the compounds in addition will have surface active properties and will simultaneously bind to the aged AChE, neutralize the negative charge of the phosphate moiety and serve as nucleophilic reagents for attacking the phosphorus. This will result in dephosphorylation and thereby reactivation of the enzyme.

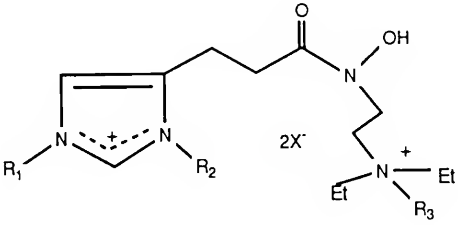
It is obvious that the success of the program is based on execution of clear design and synthesis of effective nucleophiles, and a detailed basic study of the kinetics and mechanism of the hydrolysis of diester anions. Only after the problem of diester monoanion hydrolysis is solved on the model compounds can one go to in vitro and then in vivo reactivation studies. The study is also aimed at examining the hydrolysis of neutral phosphate triesters so as to include the whole spectrum of phosphate esters and the accelerating effect of the various surfactant type nucleophilic reagents.

Design of Model Substrates and Reagents

A strong association between the nucleophile and the inhibited enzyme is an important factor in enzyme reactivation. It appears from all reactivators and antidotes so far studied (in vivo and in vitro), that structural functions such as pyridinium and imidazolium rings are involved in the binding of the nucleophile to the enzyme site that is in close proximity to the phosphorylated (inhibited) serine residue. It is reported[1] that the positively charged ring anchors on to the negatively charged carboxylate anion of the glutamic acid side chain at the active site. Therefore the inclusion of either imidazolium or pyridinium ring systems in the design of ECNR is highly desirable for structural "fine tuning" to improve maximum activity.

Hydroxamic acid ECNR

It has been shown that micellar type hydroxamates are very effective nucleophilic accelerators of organophosphates and carboxy ester hydrolysis. Kunitake et al.[18] designed some bifunctional surfactant catalysts of the imidazole type (5). These cationic surface - active nucleophilic reagents effectively catalyzed hydrolysis of p-nitrophenyl acetate and p-nitrophenyl hexanoates. On the other hand, Bodor, et al.[19] found tertiary amine containing hydroxamates such as 6 to be very effective accelerators of Paraoxon hydrolysis. It is noteworthy that hydroxylamines were used as antidotes for organophosphate poisoning prior to the inroduction of 2-PAM. *In vitro*, hydroxyl amines show high nucleophilicity since they are largely ionized at physiological pH (7.4). Based on this information, it is suggested that a class of ECNR can be designed and synthesized with the general structure as Figure 1-7.



R_1 = H , CH_3 , long alkyl group

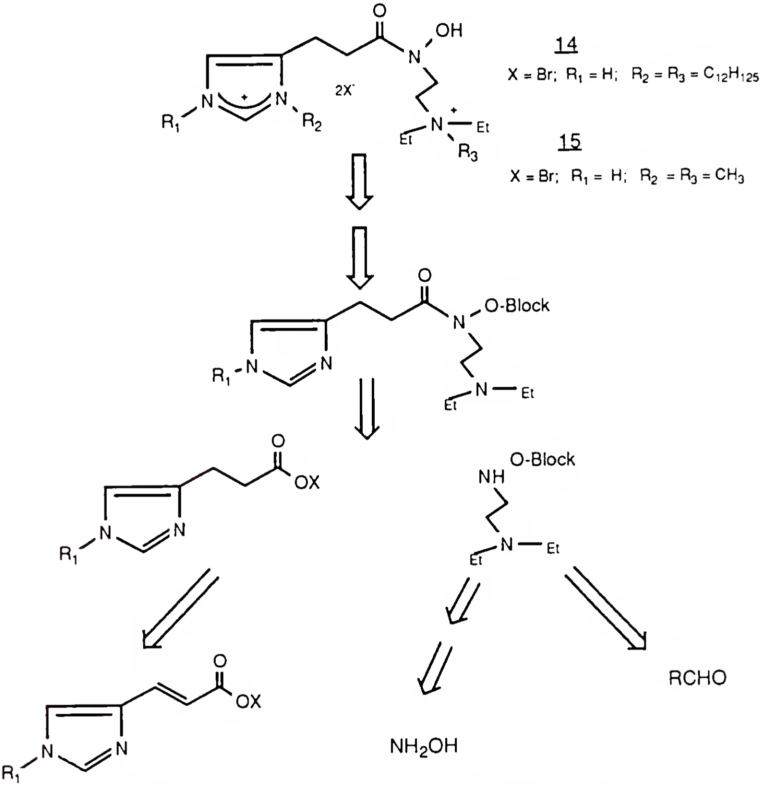
R_2 = CH_3 , C_2H_5, C_7H_{15}, $C_{12}H_{25}$, etc.

R_3 = CH_3 , C_2H_5 , or long alkyl group, etc.

X = halogen or CH_3SO_3 ,etc.

Figure 1-7: General structure of hydroxamic acid ECNRs.

The hydroxamic acid ECNRs may be synthesized starting from N and O protected hydroxylamine followed by careful N-alkylation[18] to link the imidazole moiety and the alkyl diethylamino group. Finally, alkylation of the nitrogens with the appropriate alkyl halides to create the positive segments and the selective deprotection of the benzyl group either by catalytic hydrogenation or hydrolysis should give the target hydroxamic acid ECNRs (Figure 1-8). Alternatively, the imidazole containing acids can be replaced by pyridine carboxylic acids.



14

X = Br; R_1 = H; $R_2 = R_3 = C_{12}H_{125}$

15

X = Br; R_1 = H; $R_2 = R_3 = CH_3$

Figure 1-8: Retrosynthetic scheme for hydroxamic acid ECNRs.

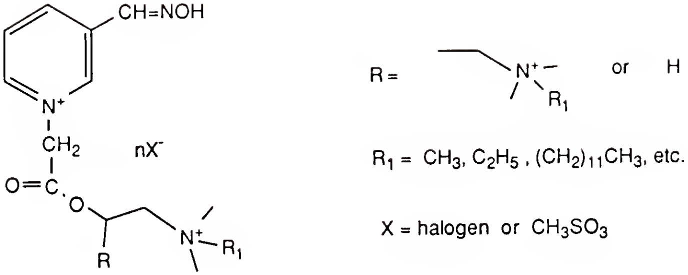
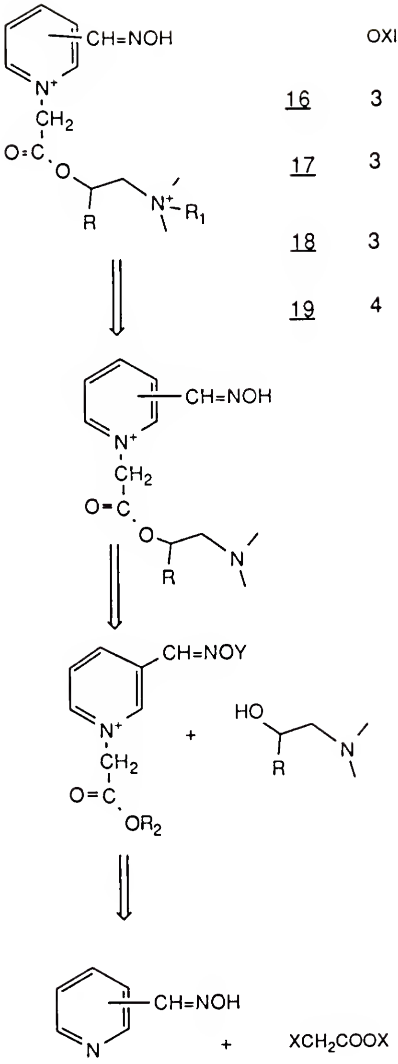


Figure 1-9: General structure of oxime ECNRs.

Oxime ECNRs

Pyridiniumoximes with the general structure as in Figure 1-9 suggested as the second type of ECNR. The structural features of this ECNR were essentially based on the oxime antidotes for organophosphate poisoning such as 2-PAM (A) and Obidoxime (B). It was proven by Epstein et al.[15] that 1-dodecyl and 1-heptyl-3-pyridiniumaldoximes are effective accelerators of hydrolysis of some neutral organophosphates. Dejon and Wolring[19] found that N-long chain alkyl 2-pyridiniumaldoxime effectively reactivated Tabun - inhibited AChE in vivo. Further, quaternary ammonium compounds such as pyridinium oximes and pyridinium salts have been shown to slow the rate of aging of Soman and Tabun - inhibited AChE[12]. Pyridinium-3-aldoximes usually are good in vitro nucleophilic models for the hydrolytic studies of organophosphates and reactivation of inhibited AChE, where as 4 and 2-oximes are generally the more potent reactivators of AChE in vivo.



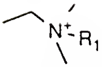
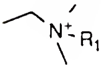
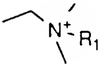
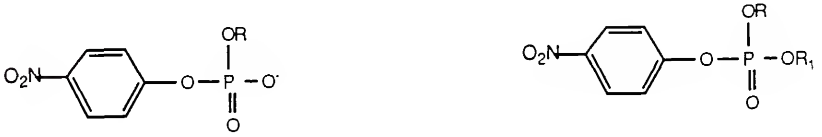
	OXIME	R_1	R
16	3	$(CH_2)_{11}CH_3$	
17	3	$(CH_2)_3CH_3$	
18	3	$(CH_2)_{11}CH_3$	H
19	4	$(CH_2)_{11}CH_3$	

Figure 1-10: Retrosynthetic scheme for oxime ECNRs

A logical retrosynthetic scheme for oxime ECNR is depicted in Figure 1-10, starting from the benzyl ether of the corresponding oxime, followed by N-alkylation of the pyridine moiety with either an α-haloacid or an α-haloacid ester of the corresponding aminoalcohol. 1-Carboxymetyl pyridinium oxime could be coupled with the aminoalcohol moiety by several methods and quaternization of the amine moieties can be achieved as the final steps. Alternatively, the pyridinium acetic acid ester function could be replaced either by transesterification with the appropriate aminoalcohol or with an amide function by reacting with an aminoamine; R_1 and R_2 can be either short, medium or long alkyl chains, or combinations; the aminoalcohol may carry a mono or bis amine function. Finally, deprotection of the O-benzyl oxime by catalytic hydrogenation would yield the target compounds. If deprotection by hydrogenolysis presents any problem, alternative routes can be found, such as trifluoroacetic acid hydrolysis.



R = O-alkyl, O-aryl, etc.

= akyl, aryl

Aged enzyme models

R and R_1 = OCH_3, $O(CH_2)_nCH_3$

= phenyl, benzyl, etc.

Triesters

Figure 1-11: General structure of aged enzyme models and organophosphorus substrates.

The use of various p-nitrophenyl alkyl phosphate or phosphonate monoanions as aged enzyme models can simulate the environment of various organophosphate inhibited aged AChE. Reaction of ECNR with these monoanion substrates will be monitored by observing the extent of product (p-nitrophenolate) formation.

The triesters (23-25) can be obtained by literature methods[26,27] from R-O-P(O)Cl_2. The diester monoanions (26-30) can be synthesized, generally by dealkylation of methyl triesters or by a direct approach (Figure 1-12). Diester anions, some carrying phospholipid analogues instead of the p-nitrophenyl moiety, can be obtained by methods described by Loew and Lacey[26]. Strict safety measures must be exercised in the handling of all organophosphate triesters carrying the p-nitrophenyl group (analogues of Paraoxon). A decontamination procedure[28] that is used for Paraoxon would be used for the waste disposal of these toxic triesters.

ECNRs with long chain alkyl groups (e.g., heptyl, octyl, dodecyl) on the hydrophilic ammonium head groups will behave as cationic nucleophilic surfactants. Rapid rate acceleration at concentrations above their CMC may be attributed to micellar properties. The micelle-substrate binding constants and electrostatic interaction between the positive groups of micelle with the negative P-O moiety at the Stern layer[29] should provide an excellent orientation for the nucleophile to access the electrophilic phosphorus atom. This would demonstrate the importance of both electrostatic and hydrophilic interaction. The nature of the micelle surface in micelle-substrate binding as well as the utility of this system as a good in vitro model for studying the specific interactions involved in reagent to aged enzyme model binding may thus be illustrated.

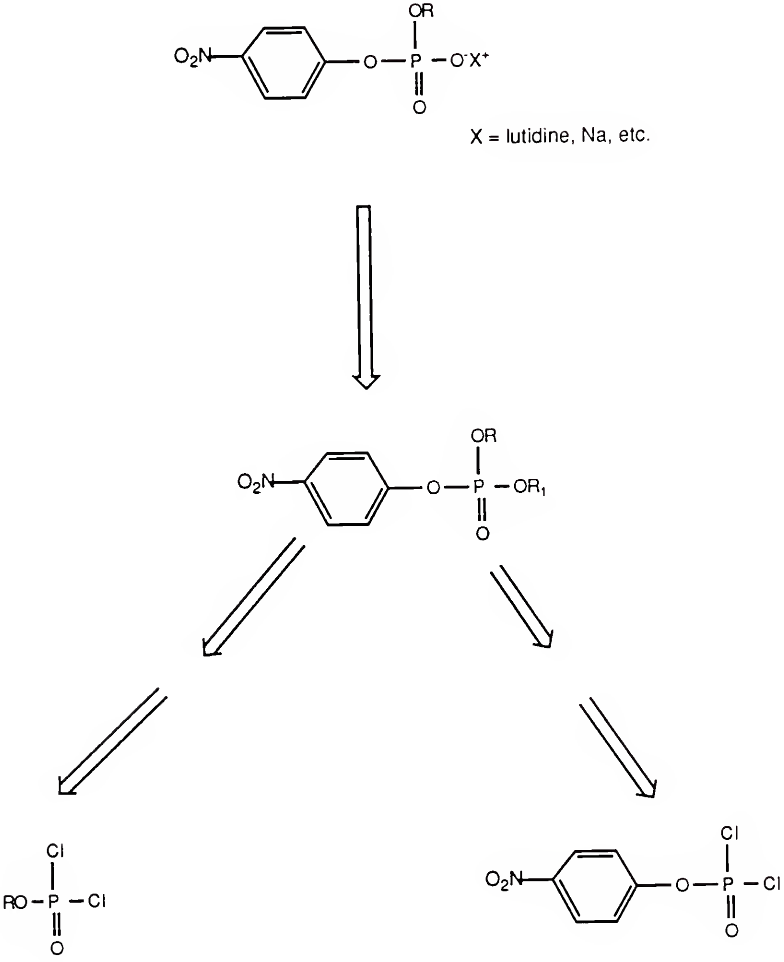


Figure 1-12: Retrosynthetic scheme for the phosphate substrates.

Physical Chemical Studies of ECNR

Kinetics

The kinetic studies of the hydrolysis of diester monoanions and neutral triesters by ECNR under various conditions would verify the efficacy of the design concept and its potential application for the reactivation of aged AChE. The activities of ECNR (~10^{-3} M) with phosphate substrates would be made at pseudo first order conditions. More detailed studies will be made under various conditions. These will include: examining reaction rates below and above critical micelle concentration (CMC), at various temperatures, at various pH's, in the presence of co-surfactants and at various concentrations. The effect of the ratio of reactants on the reaction rates should provide more mechanistic details about the reaction. The study of hydrolysis of neutral triesters by these ECNRs should clarify the accelerating effects of multicationic nucleophiles.

The kinetic measurements of hydrolysis of phosphate substrates carrying 4-nitrophenyl or 2,4-dinitrophenyl groups would be determined spectrophotometrically by following the rate of appearance of 4-nitrophenolate or 2,4-dinitrophenolate ions by monitoring UV absorbance at either 400 or 350 nm in slightly alkaline medium. It is noteworthy that hydroxide catalysis under slightly alkaline pH is likely to be negligible.[14,15] Under these circumstances interpretation of the results is less ambiguous.

Ionization constant

The effective nucleophile in the reaction is the anion of the oxime or hydroxamic acid under consideration, which indicates the role of ionization constants of ECNRs in the rate of hydrolysis. Thus, by promoting more of the ionized species without reducing its intrinsic reactivity, one could achieve a substantial rate enhancement over that observed with less ionizable cationic nucleophiles. It also has been reported[1,2,3] that there is a pK_a requirement for optimal efficacy. Oximes having pKa value <7.5 or >8.0 were found to be ineffective as reactivators in vivo. Apparently, nucleophiles with high pK_a would have only a small amount of the anion available at physiological pH to act as a nucleophile. On the other hand, if the pK_a is too low the anion will certainly be available, but will also be too weakly nucleophilic for efficacy. So the pK_a values of the ECNR help to account for the active nucleophile concentration at various pHs, and provide an indirect assessment of their nucleophilicity in in vivo conditions. The pK_a measurement of ECNRs can be made spectrophotometrically by methods described by Epstein et al.[15].

Critical micelle concentration

The CMC measurements of those ECNR carrying long hydrophobic chains would be indicative of their micellar effect on the reaction rate. A dramatic acceleration of hydrolysis will result as the concentration of ECNR reaches the CMC[29]. In general, low values of CMC parallel the enhanced reactivity of multicationic nucleophiles. These CMC measurements can be made using either a spectroflurometer[15] or surface tension apparatus[16].

Universal Decontaminant (UD)

Besides the struggle to find suitable antidotes for organophosphate poisoning it is a challenge to chemists to devise methods of destroying some of the most noxious compounds known to man, compounds which a more sane world would never produce. The structures of several of these compounds are shown in Figure 1-1 and the physiological effects of these compounds are cited in the beginning of the Chapter. Another class of chemicals that have similar military application as the organophosphates are the mustard agents.

$CH_3N\,(CH_2CH_2Cl)_2$

nitrogen mustard

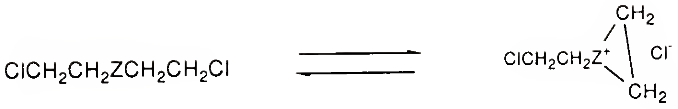
$ClCH_2CH_2SCH_2CH_3$

sulfur (half) mustard (35)

$ClCH_2CH_2SCH_2CH_2SCH_2CH_2Cl$

(HD)

Mustard agents are primarily vessicants, attacking eyes and lungs. Blisters are formed by either liquid or vapor contact. Mustards are insidious in their action; symptoms usually do not appear until several hours after exposure. Although the sulfur and nitrogen mustards have limited solubility in water at neutral pH, the small quantity which dissolves is extremely reactive. The reaction proceeds via intramolecular formation of cyclic sulfonium or immonium intermediates as illustrated in equation 1-3.



Eqn 1-3

Z = S or NR

This intermediate is highly electrophilic, and will attack compounds containing a variety of functional groups such as primary, secondary and tertiary amino nitrogen atoms, carboxylate ions, and sulfhydryl groups. With nitrogen mustards, the immonium ion, which apparently forms even in the absence of any solvent, readily attacks another molecule to form a dimer. For this reason, the nitrogen mustards are less stable than sulfur-mustard in long term storage. The physiological effects of sulfur and nitrogen mustards are similar. They act first as cell irritants and finally as a cell poison on all tissue surfaces contacted. The local action of the mustards results in conjunctivitis (inflammation of the eyes): erythema (redness of the skin), which may be followed by blistering or ulceration; and inflammatory reaction of the respiratory system. Injuries produced by mustard heal much more slowly and are more liable to infection than burns of similar intensity caused by physical means or by other chemicals.

Although chemical agents such as organophosphorus nerve agents and mustard agents have been in use for many years, only a handful of reagents have been proposed, synthesized and tested as decontaminants with some degree of success.[30,31] Potentially significant applications of such reagents and methods are in the cleanup of chemical spills such as pesticides. In military circles applications include the decontamination of equipment and personnel (dermal application) that have been exposed to chemical warfare agents, and disposal of chemical weapons.

During the past several years there have been numerous developments in this field. Decontamination of potential threat materials by rapid hydrolysis has been the major focus of such work. Because of the possibility of the use of nerve agents in military situation, particular attention has been focused upon finding an effective system to selectively hydrolyze organophosphorus compounds and sulfur mustard agents without causing any serious human toxicity.

A large number of organophosphorus and mustard compounds contain a labile halogen atom or a good leaving group such as halogen and an electrophilic center (e.g., S, P=0) susceptible to attack by OH^- resulting in displacement of the labile group.[31b] The net effect is detoxification, since the reaction products are much less dangerous and more readily disposed of safely.

Chloramine

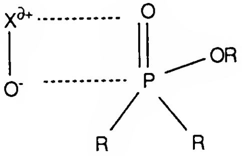
Bleach (hypochlorite) and organic bleach (N-haloamines) have been commonly utilized as decontaminants in field conditions. One such specially developed bleach is supertropical bleach ("STB')[32,33], which contains about 30 % chlorine and chloramine (chloramine T, 36)[34] bleach. "STB"[33] is used in the form of a slurry. However, it is only about 50% effective against organophosphates other than VX. This reagent suffers problems such as instability in aqueous media, and is corrosive to personnel and machinery. Chloramine T (N-bromo-N-sodiotoluenesulfonamide hydrate), is a crystalline salt, a strong oxidant in both acidic and alkaline media, and reacts readily with mustard $(ClCH_2CH_2)_2S$ to give the relatively innocuous sulfimide product.[34] A mixture of a solution containing monoethanolamine, hexylene glycol, histidine and chloramine T has been used as a non-flammable, less corrosive and low toxic decontaminant for the disposal of Soman (GD) and dichlorodimethylvinyl phosphate. Although chloramine T is a strong oxidant, it is rapidly destroyed in alkaline solution. Further, handling of chloramine T is hazardous because of the risk of explosion, which constitutes a major limitation to its use in field conditions.

Another type of reagent used by the military is agent "DS2"[32,33], a general purpose decontaminant. It consists of diethylenetriamine, ethylene glycol monomethyl ether and 2 % sodium hydroxide. "DS2" reacts with both the nerve agents and blister agents (mustard agents). Its reaction is based on hydrolysis at the electrophilic centers of the toxic reagents. The major limitations of the use of DS2 are: (a) Highly toxic vapors, (b) It is a combustible liquid (possible hazard in field conditions), (c) Protection must be worn during use, and (d) It is corrosive to metals.

The decomposition rates of organophosphate, phosphonate and phosphinate esters under various conditions are clearly of considerable importance, and effective methods for their detoxification have attracted the attention of numerous research groups over the past several years.[15,35,36] A number of these studies were aimed at micellar compounds bearing functional groups capable of catalyzing the hydrolysis of organophosphorus compounds. Epstein et al.[15] investigated the use of various oxime type nucleophilic reagents, primarily the long N-alkyl chain pyridinium oximes and obtained high micellar acceleration of hydrolysis of Paraoxon and O-ethyl-S-2-diisopropylaminoethyl methylphosphonothiolate. It has been shown by Bodor and co-workers[18] that some long chain N-alkylhydroxamic acids in the presence of cetyltrimethylammonium bromide (CTAB) significantly increase the hydrolysis of Paraoxon.[19] Some cupric ion complexes of N-alkylated N,N,N-trimethylenediamine ("metallomicelles") were shown by Menger and co-workers[35] to have a huge rate of acceleration on the hydrolysis of p-nitrophenyl diphenylphosphate (PNPDPP). Recently Katritzky and co-workers[36] developed a micellar system which consists of 2-iodoso or 2-iodobenzoic acid derivatives having long alkyl chains, and showed the potency of this micellar system against some organophosphate triesters in the presence of cetyltrimethylammonium chloride (CTAC). The catalysis by the iodobenzoic acid has been speculated as being due to the formation of anionic iodobenzoic acid species acting as the nucleophile.

The effort under this heading of the thesis was directed towards finding a universal decontaminant by a novel approach. This decontaminant should effectively decontaminate toxic organophosphates and mustard agents and itself revert to a non-toxic compound. In addition, this reagent should suit field conditions by being non-corrosive to machinery and personnel, be useful as a potential prophylactic for use against agent exposure, easy to handle, non-explosive, etc. A formulation of this reagent should also have a potential for use in vivo (dermal application). An effective decontamination system such as this would be of substantial value for both civilian and military use.

It is known that bleach (HOCl) reacts rapidly with organophosphorus compounds and mustard agents. In both cases the reactive species hypochlorite ion (OCl^-) is responsible. It has been suggested[32] that the rapid reaction with organophosphate is due to the polarized nature of $O^{\delta-}-Cl^{\delta+}$, which is therefore attracted to the $P^{\delta+}=O^{\delta-}$, group, so that a bifunctional attack is made.



X = Cl or Br

Hypochlorous acid is also known to take part in many other reactions, such as oxidation of a alcohol to ketone (aldehyde). In alkaline medium OCl^- ion is notably more nucleophilic than OH^- ion, and attacks electrophilic centers in the reaction medium. It is noteworthy that HOCl has a pK_a around 7.4; therefore in aqueous medium there will be adequate nucleophile concentration (OX^- ion) available for reaction. Epstein et al.[38(a)] showed hypochlorite ion as one of the most effective catalysts for the hydrolysis of Sarin and O,O,S-triethylphosphonothiolate under mild alkaline conditions. Epstein also showed that these reactions involve a direct substitution of the fluorine atom by hypochlorite anion. This process determines the rate of overall reaction since it is followed by a rapid hydrolysis of the intermediate. The effectiveness of OCl^- over OH^- was greater than could be accounted for simply by the increased nucleophilic power bestowed by the Cl upon the O.

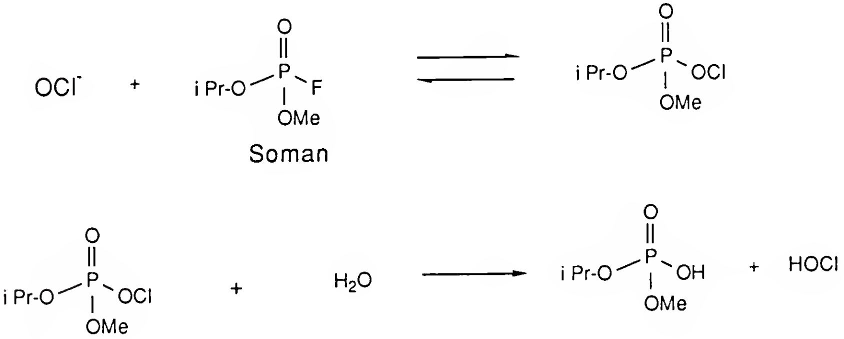


Figure 1-13: Reaction of hypochlorite anion with Soman.

The mechanism of detoxification of mustard agent by hypobromous acid appears[38] to be as described Figure 1-14. The oxidation of S to $S \rightarrow O$ dramatically reduces its alkylating potency. Further hydrolysis of the product results in complete destruction of the functional moieties.

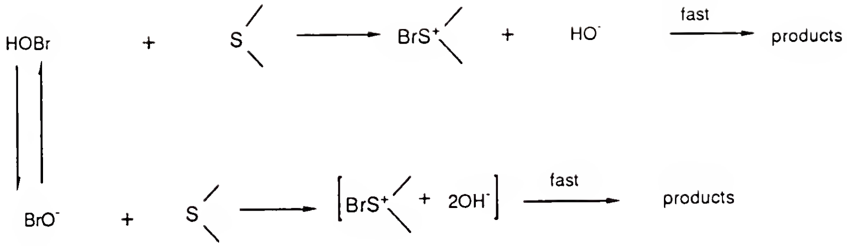
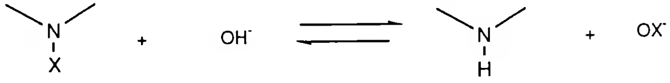


Figure 1-14: Reaction of mustard agent with hypobromous acid.[38]

N-Haloamines have been used as positive halogen (X^+) sources in various reactions. They have a labile $N^{\delta-}$-$X^{\delta+}$ bond that produces reactive O-X^- (hypohalide ion) under alkaline conditions. Due to their increased stability as positive halogen sources they are better decontaminant formulations than is conventional bleach (e.g., NaOCl, or $Ca(OCl)_2$). Although N-haloamines have been used in the decontamination process,[31,33] none have proven satisfactory as a safe universal decontaminant for organophosphates and mustard agents. Consequently, they have no practical value for in vivo or in vitro use.

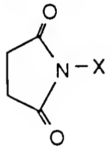


Eqn 1-4

X = Cl or Br

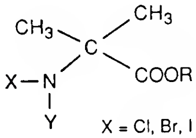
N-Haloamines

It has been shown by Bodor et al.[39] that some novel N-chloramines are effective as a non-toxic and stable source of positive chlorine (Cl^+), and some of these compounds are effective as antimicrobial agents. N-Bromosuccinimide (NBS)[37, 40] is known as a stable and versatile "positive" bromine reagent among the number of the N-halo-compounds. Only a few N-halo derivatives of amides and amino acids have been shown to be useful "positive" halogen sources. This has been attributed to the almost non-polar nature of their N-halogen bonds as well as the favorable geometric arrangements which exist between the N-halogen bond and the carbonyl functions. In general, the lower the polarity of the N-X bond, the more stable the N-haloamine. The haloamine bond has properties different from those of covalent C-X bonds. For many properties, it is convenient to regard chlorine (or bromine) when bonded to a nitrogen as "positive source".



X = Br, Cl

37

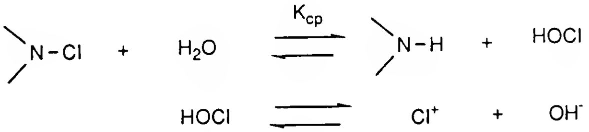


X = Cl, Br, I
Y = halogen, H
R = alkyl moiety

38

In the effort to find a stable and non-corrosive reagent Bodor et al. designed and synthesized various chloroamides based on N-halogen bonds of low "chlorine potential". The chlorine potential is defined as pk_{cp}, $pk_{cp} = -\log k_{cp}$ where k_{cp} is the equilibrium constant[41] for the hydrolysis of N-chloro compounds to yield hypochlorous acid (Eqn 1-5).

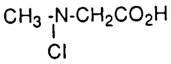
Direct determination of the equilibrium constant (k_{cp}) values is only possible in a few cases since the equilibria are usually so far to the left that direct measurement is not practicable. In such cases it is more convenient to measure the equilibrium constant, K_{equ} for chlorine exchange reactions and relate the results directly to hypochlorous acid. The K_{equ} value is then used as an experimental measure of the polarity of the N-Cl bond. A high K_{equ} value is probably due to the polarization of the N-Cl bond in the N-chloro derivative, and changes. This polarization changes from $N^{\delta-}$——$Cl^{\delta+}$ for derivatives of strong bases to $N^{\delta+}$——$Cl^{\delta-}$ for derivatives of weak bases.



Eqn 1-5

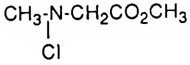
The former type of polarization would destabilize the molecule with respect to separation of $Cl^{\delta+}$, whereas the latter type polarization would stabilize the molecule. Bodor et al.[42] studied the polarization of N-Cl bonds of structurally different N-chloramines by photoelectron spectroscopy and compared the polarization with the basicities of the parent amines. Their investigation showed that the polarity of N-Cl bonds corresponds to the basicity of the parent amine and chlorine potential of the N-chloramine.

A number of "low chlorine" potential N-chloramines and N-chloramides containing low polar N-Cl bonds have been reported by Kaminski et al.[39,43] The chlorination of α-amino acids and the corresponding N-chloro derivatives were investigated for use as low chlorine potential compounds and positive chlorine. Their results elucidated the factors that significantly influence the stability of these N-chloramines and other low chlorine potential N-chloramines in general. N-Chloramines of α-aminoacids, including those even the N-methyl derivatives (39 and 40) containing α-hydrogens, decompose rapidly via an elimination (dehydrohalogenation) pathway. This decomposition is sometimes explosive (40). Decomposition pathways through decarboxylation were also observed with carboxylates.[43]



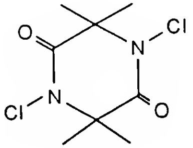
$CH_3\text{-}N(Cl)\text{-}CH_2CO_2H$

39



$CH_3\text{-}N(Cl)\text{-}CH_2CO_2CH_3$

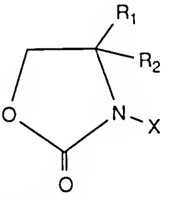
40



41

On the basis of these observations, 1,4-dichloro-2,2,5,5-tetramethyl-3,6-piperazinedione (41) was designed and studied.[43] The incorporation of geminal dimethyl substitution at the α-carbon and a cyclic amide (lactam) linkage resulted in greater stability. In addition to the structural requirements, a synthetic amino acid was chosen as the precursor, which should ideally exhibit an inherently low toxicity. These types of low chlorine potential N-chloramines are of interest as "soft" positive chlorine sources for use in vivo (eg., skin) These N-chlorinated α-amino butyric acids and their derivatives exhibited a high degree of antimicrobial activity by inhibiting the bacterial growth due to inhibition DNA, RNA and protein synthesis without readily being deactivated by extraneous reaction.[44]

Based on the low polarity of the nitrogen-chlorine bond and the soft nature of the N-chloramines, the N-halo-4,4-dimethyl-2-oxazolidinone system (43) was developed as a cyclic derivative of 2-amino-2-methyl-1-propanol, a compound of low toxicity.[44,45] The ideal proximity between the amino and hydroxyl groups in 2-amino-2-methyl-1-propanol lead to the investigation of the 2-oxazolidinone system as a precursor for a low chlorine soft N-chloramine. This system contains specific structural requirement, essential for optimizing the stability and reactivity of the nitrogen-chlorine bond; that is, the gem-dimethyl substitution at the carbon adjacent to the nitrogen-halogen bond.



X = Cl, Br, H

$R_1 = R_2 = CH_3$

X = H (42)
X = Br (43, NBO)

Bodor and Kaminski[45] examined a series of N-bromo derivatives of the 2-oxazolidinone system and tested their ability to serve as versatile brominating agents with respect to N-bromosuccinimide (NBS, 37). Out of the many 4,4-dialkyl derivatives, 3-bromo-4,4-dimethyl-2-oxazolidinone (NBO, 43) proved to be by far the most stable. The polarity of N-bromine bond in NBO is low, primarily as a result of the electron donating inductive effect of the adjacent methyl groups, and resonance effects of the oxygen atom adjacent to the carbonyl group.

NBO was also found to be equivalent or better than NBS as a brominating and oxidizing agent. The term "bromine potential" in analogy to the "chlorine potential" was used to explain the low bromine nature of NBO with respect to NBS. The bromine potential was defined as the equilibrium constant (K_{equ}) for the reversible reaction between NBO and NBS.

Bodor and Kaminski[45] determined the equilibrium constant of this process and related that to relative polarity of the N-Br bond with respect to that of NBS. A value less than 1.00 is indicative of a low polarity N-Br bond with respect to NBS. Low bromine compound such as NBO showed a value $k_{equ} = 0.34$. Although NBO satisfies all criteria of an effective soft low bromine potential N-bromoamine, its low water solubility (0.5%) seriously hinders its use in aqueous medium.

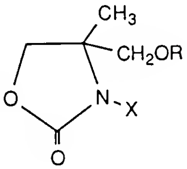
Specific Aims (N-Haloamine Decontaminants)

Non-corrosive N-bromoamines that are structurally similar to the 2-oxazolidinone system with additional features to increase aqueous solubility without reducing the stability and reactivity would serve as "soft" Universal Decontaminants for organophosphate and mustard agents. Some of the specific compounds sould exhibit micellar properties and some would be designed to yield increased active bromine/per mole. In addition to the chemical use of these compounds, some should possess potential in vivo (dermal) application. A detailed investigation based on clear design and synthesis of N-bromoamines, examination of their stability under various conditions, and kinetic study of hydrolysis of organophosphate and mustard models is essential.

Design

2-Oxazolidinone decontaminant system

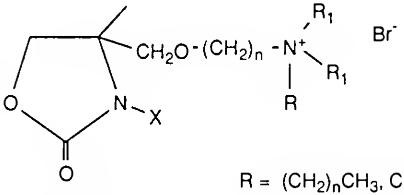
Based on avilable information it is envisaged that a careful functionalization of 4,4-dimethyl-2-oxazolidinone should lead to the "fine tuning" of the 2-oxazolidinone system for a Universal Decontaminant (UD). Functionalizing one of the methyl groups of the gem dimethyl α-carbon would not detract from the stability requirement of having a dialkyl-substituted carbon α to the N-bromine bond.



R	X
H	H (44), Br(45)
SO_3H	H (46), Br (47)
CH_2CO_2H	H (48), BR (49)
$(CH_2)_nNEt_2$	H (50)

The electron donating inductive effect of the 4,4-dimethyl groups in providing a less polar N-Br bond is unlikely to be altered by hydroxymethylation of one of the methyl groups. However, the hydroxyl group would certainly increase the polarity of the molecule. The aqueous solubility of the 4-hydroxymethyl-4-methyl-2-oxazolidinone (44) and its N-bromo derivative can be manipulated by derivatizing the hydroxyl group with carefully selected functional groups. For example, conversion of (44) to a sulfonate (46) sould dramatically increase the aqueous solubility of 46 and its N-bromoamine (47). A sodium salt of 47 would be an ideal candidate, with all requirements for a Universal Decontaminant. Another "soft" analogue will be the dicarboxylic acid ester of (48). The extension of chain length of 49 by more than about six carbons would add lipophilicity to the molecule and thereby provide a unique solubility property. The attachment of an alkylamine moiety to the oxazolidione system was intended to provide a structural frame (50) that can be transformed to various quaternary forms (51 and 52), and some of the quaternary systems with long alkyl chains are expected to show micellar properties.

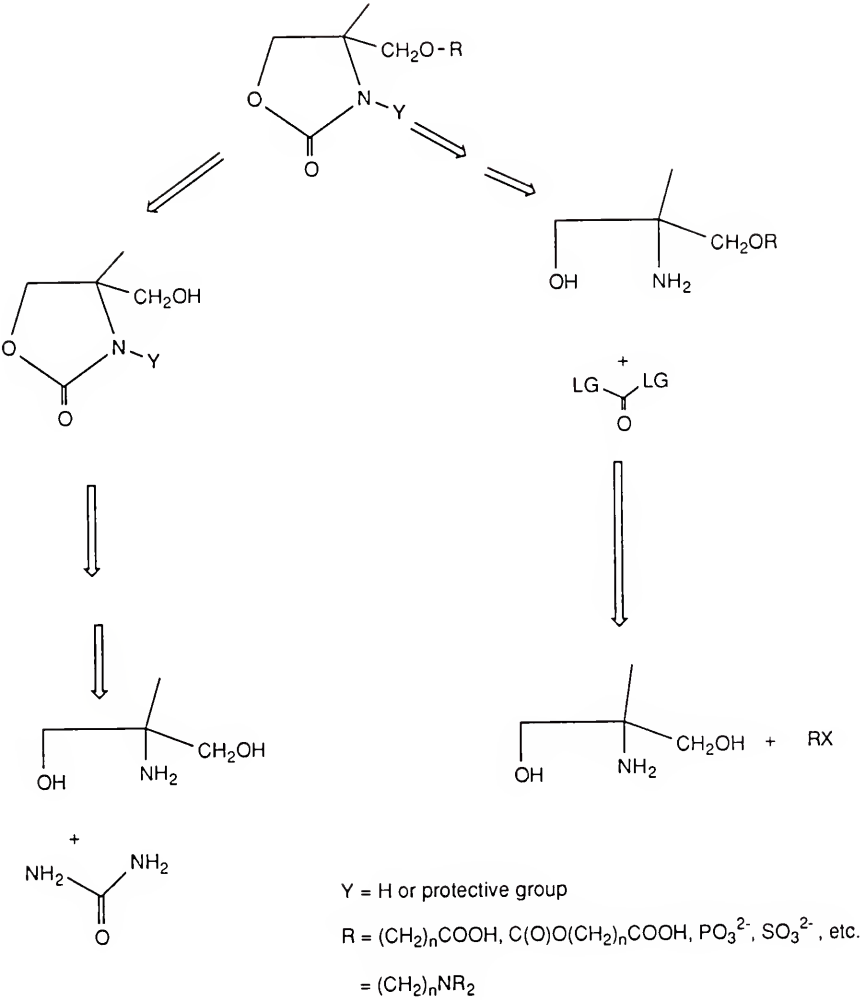
The compatibility of various cationic surfactants (e.g., 1-n-dodecyl-3-pyridiniumaldoxime chloride (2-PAD-Cl), hexadecyltrimethyl ammonium bromide (CTAB), 2-n-hexadecyldimethylammonium-1-ethanol bromide) with various N-haloamines and their activity in deactivating chemical agents has been examined.[46(a)] The studies showed that only some cationic ionic surfactants are compatible in a premade mixture. However, this system significantly increased the reactivity of NBO against chemical agents by formation of Br_3^- species, which was subsequently captured by the cationic surfactant micelle. Based on these findings a covalent attachment of dodecyl dialkylammonium ethanol ether of 52 should be effective as a cationic micellar N-bromoamine.



51-52

$R = (CH_2)_nCH_3, CH_3$
$R_1 = CH_2CH_3$ or CH_3

X= H or Br



Y = H or protective group

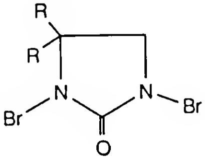
R = $(CH_2)_nCOOH$, $C(O)O(CH_2)_nCOOH$, PO_3^{2-}, SO_3^{2-}, etc.

= $(CH_2)_nNR_2$

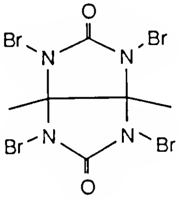
Figure 1-15: Retrosynthetic scheme for the derivatives of 4-hydroxy-4-methyl-2-oxazolidinones.

Increased active bromine/molecular weight

A somewhat related brominating agent which has been used in several synthetic procedures with various degrees of success is 1,3-dibromo-5,5-dimethylhydantoin[47], which yields two positive bromine per molecule. However, compound (53) suffers the major disadvantage of containing the α-hydrogens, and hence contains N-bromo bonds of relatively high polarity. Thus, compounds of the type 54 are the objectives. A structure such as 54 will afford four active bromides per molecule, thereby increasing the available reactive species per mole of reagent by four fold.



53



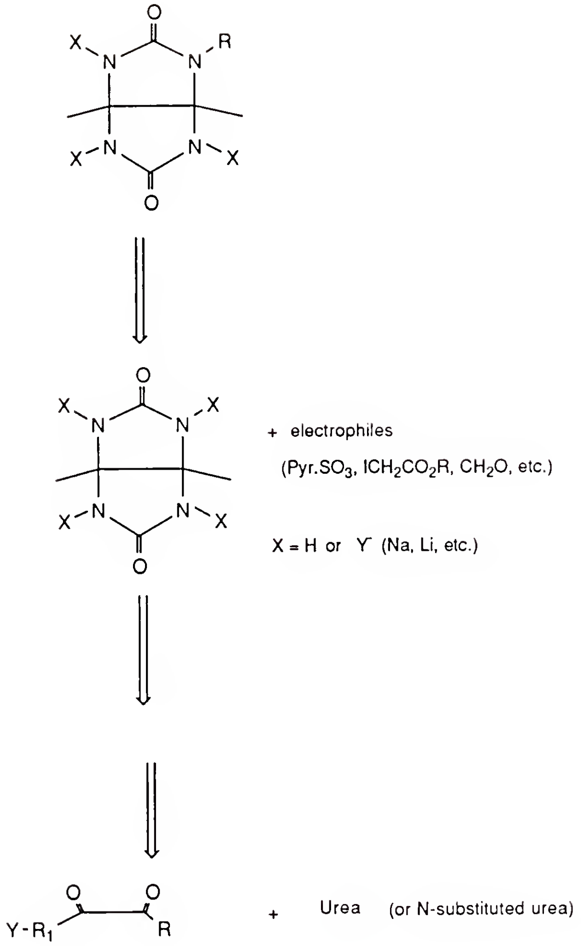
54(b)

It is necessary, from a stability standpoint, that there should be no hydrogens on the carbon α-to the N-bromo bond in order to prevent dehydrohalogenation. Rapid dehydrohalogenation from more than one part of the molecule could result in an explosion. Once the active bromine content per mole has been increased, the next approach will be the insertion of solubilizing groups such as hydroxymethyl, carboxylate and sulfonate moieties on to one of the nitrogen of the glycouril system. These substituents should dramatically increase the solubility of N-bromo glycouril system in polar organic solvents and water whilst retaining three active Br^+ per molecule.

Synthesis

The 2-oxazolidinone system can be prepared by methods similar to the one used for protection of carboxyl functions in carboxylic acids.[47] First, an appropriate ethanolamine with ideal proximity between amino group and hydroxyl group has to be found. Condensation of 2-amino-2-methyl-1,3-propanediol with urea has been reported to give(44) in excellent yield.[48] This hydroxymethylated oxazolidinone could be converted to its bromo derivative. However, under N-bromination conditions oxidation may occur at the hydroxymethyl site. Reacting (44) with a pyridine-sulfur trioxide complex should yield the desired sulfate derivative. The corresponding sodium salt of the sulfate may be obtained either by treatment on an ion-exchange column (Na^+ form) or by reacting with one equivalent of sodium hydroxide.[49]

Carboxylic acid derivatives of 46 may be obtained by reaction with a dicarboxylic acid anhydride (e.g., succinic anhydride) or by treatment with monoacyl chlorides of dicarboxylic acids (e.g., malonyl chloride) in a suitable solvent. Cyclic anhydrides with various chain lengths would provide a series of carboxylate derivatives of 44 with various solubility properties. Reaction of the bis sodium salt (or lithium salt) of 44 with one equivalent of chloroethyl diethylamine under mild conditions would provide the corresponding O-ethyl diethylamine compound. Quaternization of this free amine with an alkylbromide such as dodecyl bromide would yield the precursor for a micellar N-bromo oxazolidinone system (52).



+ electrophiles

(Pyr.SO_3, ICH_2CO_2R, CH_2O, etc.)

X = H or Y^- (Na, Li, etc.)

+ Urea (or N-substituted urea)

Figure 1-16: Retrosynthetic scheme for the dimethylglycouril systems.

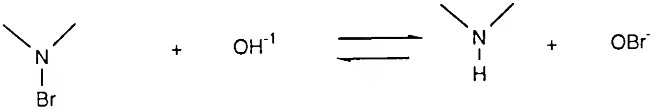
Dimethylglycouril (3a,7a-dimethyltetrahydroimidazo [4,5-d] imidazole-2,5-dione) (54) and its analogues would be synthesized by condensation of the appropriate α,α-diketone with urea.[50] Using an equimolar ratio of formaldehyde with (54) it should be possible to prepare mono N-hydroxymethyl dimethylglycouril under controlled conditions. Following the success in monohydroxymethylation of glycouril further derivatization with sulfur trioxide-pyridine complex should provide mono N-methylsulfate dimethylglycouril, which is expected to have high water solubility.

The synthetic strategy involves preparation of the monoanion of dimethylglycouril in situ either by reacting with sodium hydride (NaH) or lithium diisopropylamide (LDA). Subsequent reaction with appropriate electrophiles (e.g., XCH_2CH_2OH, $BrCH_2CO_2Et$, SO_3-pyridine) would provide a flexible solution for the synthesis of monosubstituted glycourils. Use of electrophiles that carry long alkyl chains would yield a product that could have emulsifying properties. Methods for the N-bromination of various amines, amides and amino acids are well illustrated by Bodor, et al.[43-45] Generally bromine/aqueous sodium hydroxide solution is used under cold conditions. Since all designed N-bromoamines are expected to have lower reactivity (low bromine potential) than N-bromosuccinimide, bromination of the N-H bond could possibly be achieved by reacting with NBS. Most of the brominated amines are easily isolable as precipitates from the reaction mixture at -5 to 0°C or by solvent extraction. However, highly water soluble N-bromo products may be isolated from the aqueous phase by freeze drying techniques (lyophilization).

Physical Chemical Studies of N-Bromoamines

Bromine content

Active bromine content of the N-bromoamines would be analyzed principally by an iodometric method using standardized sodium thiosulfate solution.[39,51] Active bromine content can also be determined spectrophotometrically by recording the UV absorbance at about 350 nm in mildly basic solution.



Eqn 1-8

Stability studies

The stability measurement of N-bromoamines at various conditions is an integral part of the evaluation process. These measurements will also render kinetic information about factors influencing the stability of the compound. The effect of pH of the medium, concentration of the compound, and temperature will be examined. The decomposition half life of the compound can be measured by determining the positive bromine content iodometrically or spectrophotometrically over a period. The spectrophotometric method involves monitoring the change of absorption of the initial N-bromoamine or the change in hypobromite ion (OBr^-) production at 350 nm. It has been pointed out that kinetic rate constants are independent of methods used to determine bromine content.[44]

The decomposition of N-haloamines in aqueous solution generally follows a first order kinetic process.[39] Therefore, the apparent rate constant can be characterized from the reaction half-life. Characteristic UV absorption spectra would be used to determine product composition and decomposition products. Stability studies will also be extended to determine the compatibility of external surfactant - N-bromoamine mixtures.

Bromine potential

The "bromine potential", defined as the equilibrium constant (K) would be determined spectrophotometrically from the reversible reaction between N-bromoamine and succinimide. The value of K_{equ} will be calculated by using a kinetic equation derived by Kaminski and Bodor[45] from the absorbance data at a wavelength where the corresponding free amine or NBS has no appreciable absorption. The observed equilibrium constant will relate to the polarity of the N-bromine bond and to the stability data.

Kinetics

The major objective of the effort in this research is to verify the effective reactivity of each of the N-bromoamines synthesized or formulations thereof against organophosphate and mustard model compounds. The kinetic result obtained from these experiments should correspond to the potency of novel N-bromoamines in decontaminating the chemical agents. The model compound for a phosphate ester to be used in the kinetic studies is diethyl p-nitrophenyl phosphate (Paraoxon). Paraoxon is generally more stable towards hydrolysis than is the agent G (GD (Soman)), while it is somewhat less stable than the agent V (VX).

However, due to the advantages offered by the ease with which its cleavage can be followed by monitoring the appearance of the p-nitrophenolate ion, it was preferred over the other possible model, DFP. The model compound for the mustard agent (bis-(2-chloroethyl)-sulfide) is the corresponding "half mustard" (2-chloroethyl-ethyl sulfide).

The destruction efficacy of N-bromoamines (~10^{-2} to 10^{-3} M) against Paraoxon (~$5x10^{-5}$ M) would be determined from the pseudo first order-rate constant for the hydrolysis at various pHs, at different buffer concentrations, and at different reagent concentration, at various temperatures. A rational examination of the kinetic data with the stability values will allow prediction of the optimum reaction conditions needed for a maximum efficacy. The reaction rate constants would be calculated either by half life method or by initial rate method from the absorption data recorded over at least 4 half lives.

The kinetic studies of the destruction of 2-chloroethyl-ethylsulfide by N-bromoamines will be made by assaying the mustard agent concentration in the reaction mixture by a gas chromatographic method, at different intervals. A G.C instrument with a flame ionization detector will be utilized for such determination.

CHAPTER II
RESULTS AND DISCUSSIONS

Synthesis of ECNR and Phosphate Models

Synthesis of Hydroxamic Acid ECNR Compounds

A scheme (Figure 2-1) using the route executed by Kunitake, et al.[18] was initially outlined for the preparation of hydroxamic acid target compounds. This route begins from benzyl benzohydroxamate, at N and O protected (acylated) hydroxyl group, and requires N-monoalkylation and subsequent deprotection of the N-acyl group in order to introduce the acyl group of interest. Benzyl benzohydroxamate 56 was prepared[52] by the reaction of the potassium salt of benzohydroxamic acid 55 with benzyl bromide in the presence of potassium carbonate in 60% yield. The potassium salt of the hydroxamic acid was obtained from commercially available benzohydroxamic acid using ethanolic potassium hydroxide. Using a similar method to that of Kunitake et al.[18,53], benzyl benzohydroxamate was reacted with sodium hydride in DMF to generate the corresponding anionic salt, and the subsequent attack with 2-chloroethyl-1-diethylamine gave the N-alkylated benzyl benzohydroxamate. Owing to the instability of the free amine form of the electrophile, 2-chloroethyl-1-diethylamine was generated at mild conditions prior to its use. The N-alkylated product (57) was obtained in widely varying yield (30 - 60%), which similar to that observed by Kunitake et al.[53] in some N-alkylation (or acylation) reactions of benzyl benzohydroxamate.

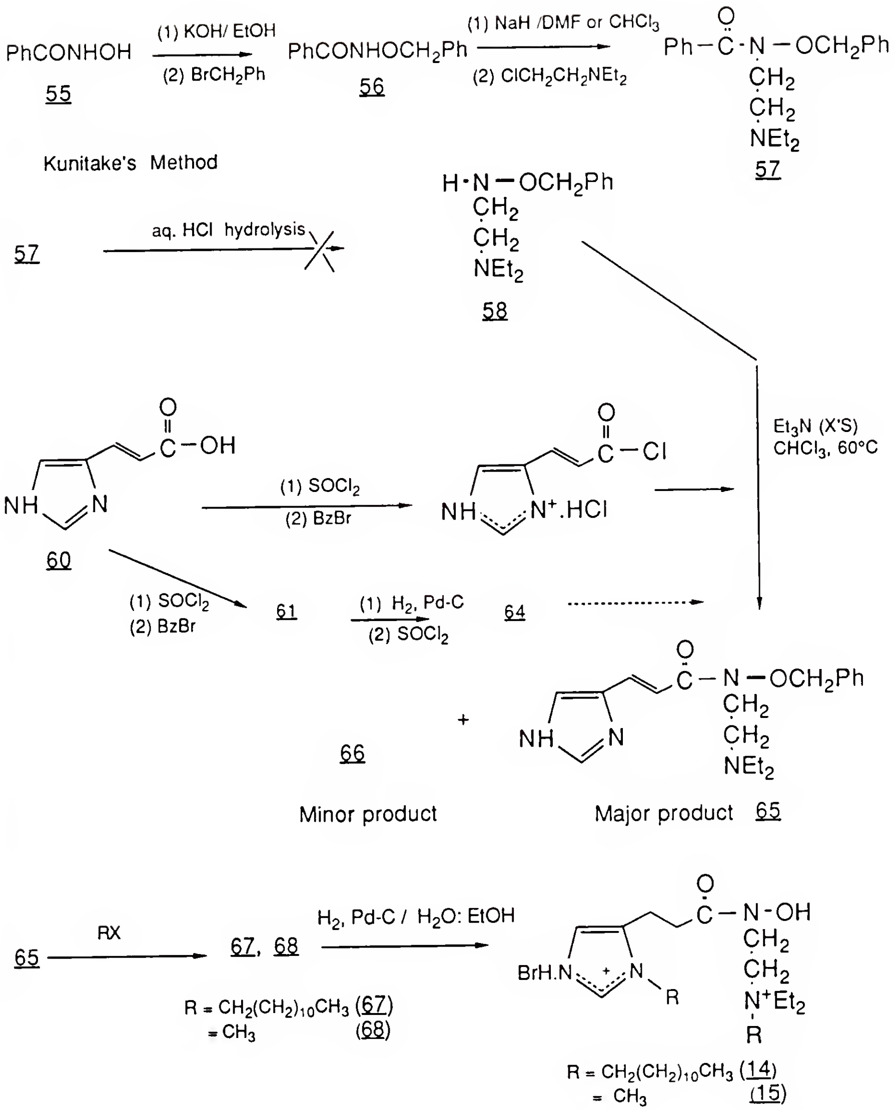


Figure 2-1: General synthetic scheme for hydroxamic acid ECNRs.

Kunitake's method [18,53] of removal of the benzoyl group from 57 under hydrolytic conditions to yield N-(β-diethylaminoethyl)-O-benzyl hydroxyl amine (58) was not successful. This method of removal by acidolysis under various hydrolytic conditions gave no trace of 58, but gave a debenzylated product mixture which spectrally (IR, ^{1}HNMR) corresponded to the N-(β-diethylaminoethyl)benzohydroxamic acid [$PhC(O)NH(OH)CH_2CH_2N(CH_2CH_2NEt_2$]. It appears that under the hydrolytic conditions employed the benzyl group is selectively removed from 57 instead of the benzoyl moiety, a result different from that observed [18] with N-dodecyl benzyl benzohydroxamate under similar conditions.

Acylation of 56 with imidazole propionoyl chloride prior to N-alkylation with chloroethyl diethylamine was an ambitious attempt at not having to go through 57 in the hydrolytic cleavage of the benzoyl group. However, acylation of 56 using an adopted literature [18] procedure failed to yield the corresponding N-acyl-O-benzyl benzohydroxamate, and instead resulted in an complex product mixture.

A search for alternative routes[54,55] to N-substituted hydroxylamines that avoids hydrolytic reactions led to the finding of a direct and simple approach (Figure 2-2) to prepare 58. The reaction involved a one step alkylation of O-benzylhydroxylamine with 2-chloroethyldiethylamine in the presence of a controlled amount of triethylamine to afford 58 in moderate yield (≈ 60%). The compound 58 was isolated as the major product from the reaction oil by vacuum distillation, and was characterized by ^{1}H NMR and mass spectrum. A high boiling fraction that was isolated from the reaction oil accounted for less than 5% of the total yield and was identified as the dialkylated O-benzylhydroxylamine

(59) on the basis of its ^{1}H NMR spectrum. Neither of these products (58 and 59) are reported in the literature. Wawzoneck and Kempt [54] have found that N-alkylation of hydroxylamine always yields a mixture of N,N-dialkylhydroxylamine and N-alkylhydroxylamine irrespective of the ratio of the alkyl halide and hydroxylamine employed. The optimum conditions worked out to provide increased yields of 58 were (a) use of not more than 2.1 equivalent triethylamine, (b) use of 1.1 molar ratio O-benzylhydroxylamine/2-chloroethyldiethylamine hydrochloride and (c) mild reflux conditions.

Other routes to make 58 such as direct monoalkylation of free hydroxylamine (HN_2OH) were explored. Reaction of NH_2OH with 2-chloroethyldiethylamine gave a product mixture containing largely (>80%) the dialkylated product. This was not totally unexpected, owing to the unprotected nature of hydroxylamine and the high reactivity of the electrophile.[54] The synthesis of O-benzyl-N-(β-diethylaminoethyl)-3-(4-imidazolyl)propeniohydroxamate (65), the major synthon to the hydroxamic acid target molecule, presented enormous difficulties. Addition of the acyl chloride of urocanic acid (3-(4-imidazolyl)propenoic acid) to 58 in the presence of excess base (triethylamine or pyridine) under various conditions gave a product mixture containing little or no 65. Use of sodium hydride to generate the anion of 58 followed by the addition of acylchloride 60 gave a non-olefinic compound which showed two β-diethylaminoethyl moieties intact. It is not unreasonable to suppose that the anionic 58 could add across the double bond of an α,ß unsaturated hydroxamate [56]; this may well occur on formation of 65 in the reaction medium.

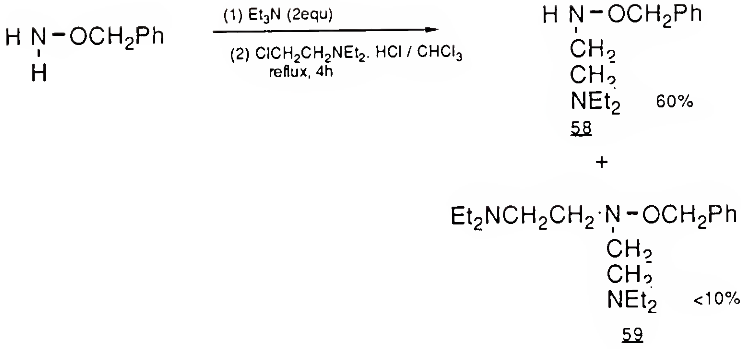
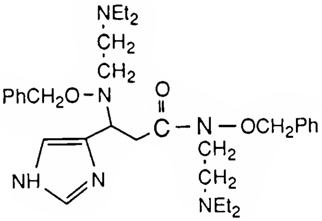


Figure 2-2: Direct synthesis of N-(β-diethylaminoethyl)-O-benzyl hydroxylamine.

After a series of unsuccessful attempts, a method utilizing inverse addition of the 3-(4-imidazolyl)propionyl chloride to a well stirred solution of 58 in the presence of ~ 10 molar excess of triethylamine or pyridine gave the desired coupling to yield 65 along with other minor components. The major component (R_f 0.34), the desired product 65, was isolated after repeated flash chromatography on silica gel in 15% yield. The structure of 65 was confirmed by mass spectral, ^{1}H NMR and C.H.N data. The ^{1}H NMR (300 MHz) spectrum (Figure 2-3) shows 65 to be solely in one isomeric form, and trans identity was established based on the trans coupling constant[57] (J_{ax} ≈ 15 HZ) of the alkenic doublets. A component with lower R_f (0.18) than 65 was also isolated as the side product during the chromatographic separation, and accounted for about 5% of the crude yield. This side product was assigned structure 66 on the basis of spectral data, (M+1) peak at the expected value of 565. The ^{1}H NMR spectrum showed the absence of akenic proton signals and accounted for two diethylaminoethyl moieties. Mechanistically, addition of 58 to 65 in a Michael fashion should yield 66.

Rajendra and Miller[56] have utilized Michael type addition of amino groups to ß, -unsaturated O-acylhydroxamates to form ß-lactams with α-amino substituents. Allowing the reaction mixture to age over 48 hours has been shown to increase the yield of side product (65) from 5% up to about 10% of the total yield. On the other hand, use of short reaction time (less than 4-5 hours) resulted in a low yield of 65 with incomplete reaction.



Micheal adduct 66

The N-alkylation of 65 with alkyl halides to give mono or dialkylated derivatives was not as straightforward as expected. The choice of solvent (or solvent system) was crucial for the completion of reaction of 65 to its dialkyl alkylated derivative 67. In general, less polar solvents are expected to favor N-alkylation of more basic nitrogen (trialkyl amine) over less basic nitrogens (aromatic heterocycles). However, non-polar solvents such as ethyl acetate and chloroform with excess or one equivalent of dodecyl bromide gave a mixture of di- and mono-dodecyl quaternary salts of 65. Reaction in chloroform-acetonitrile 3:1 gave an amorphous solid of dialkylated 65 (67) in 63% yield.

Since monoalkylation of 65 could not be achieved without having some degree of dialkylation, attempts to make the monododecyl salt of 65 were reverted towards synthesis of the didodecyl salt. In a similar attempt, treating 65 with excess of methyl iodide in chloroform-acetonitrile 2:1 in a pressure bottle afforded 86% yield of the dimethyl analogue of 65 (68). Purification of compounds 67 and 68 by solvent recrystallization was not successful, and the cationic nature of these compounds precluded any preparative chromatographic purifications. In spite of these problems, the products were purified by a series of solvent (petroleum ether, diethyl ether and ethyl acetate) washes. The pure samples of 67 and 68 showed satisfactory C. H. N. analysis and ^{1}H NMR.

A few options are cited in the literature for the removal of O-benzyl groups, for example, hydrogenation over Pd-$SrCO_3$[53] and acidolysis[58] selectively remove O-benzyl group without cleaving N-alkyl groups. A trial hydrogenation of 67 over 2% Pd-$SrCO_3$ showed no sign of reaction. Hydrogenation over 5% Pd-C in absolute ethanol over 3 days at 30 psi hydrogen pressure gave only partial deprotection.

Finally, removal of the benzyl group and the reduction of the double bond of 67 and 68 were accomplished successfully by hydrogenation over 5% Pd-C in ethanol-water 6:8 over 12 hours to give the final compounds (hygroscopic solids) 14 and 15. It is noteworthy that simultaneous deprotection and reduction in the final step allowed the elimination of three additional steps that were originally planned. These additional steps would have involved conversion of 3-(4-imidazolyl)propenoic acid (60) to 3-(4-imidazolyl)propionic acid (64) instead of the direct use of acylchloride of 60 with 58 to yield 65.[60]

The mass spectral data of the final products were obtained using fast-atom bombardment (FAB) in a glycerol matrix. The conventional type of electron ionization methods (e.g., low eV EI) often fail to show crucial mass spectral data for multiquaternary salts. The FAB techniques have proven[59] to be the method of choice for unveiling molecular ion peaks of quaternary ammonium compounds. The mass spectrum of 67 showed a strong peak (M^+) at the expected value of 680. However, the mass spectrum of 68 showed no molecular ion peak but instead showed a peak at 284 corresponding to M-N^+Et_2(Me). The hydroxamic acids 14 and 15 showed strong (M-1) peaks at the expected values 591 and 283 respectively, corresponding to the dialkylated products.

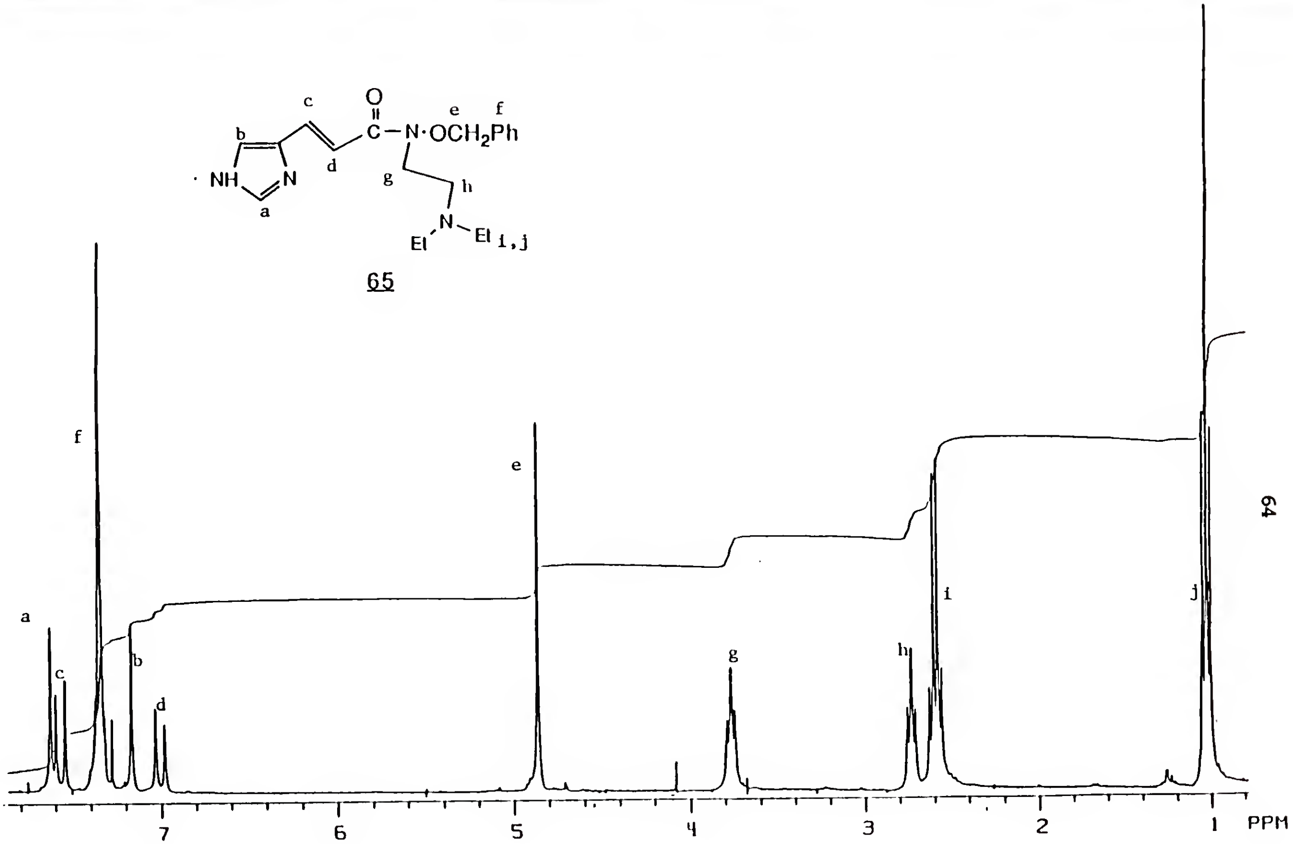
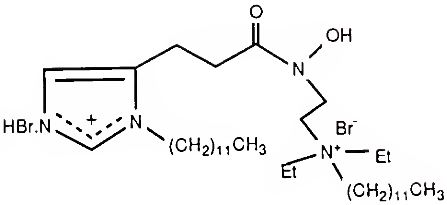
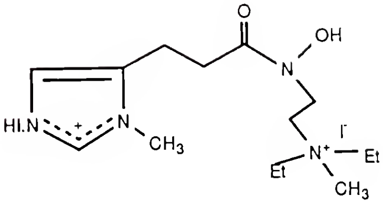


Figure 2-3: The ^1H NMR spectrum (300 MHz) of 65 in $CDCl_3$.



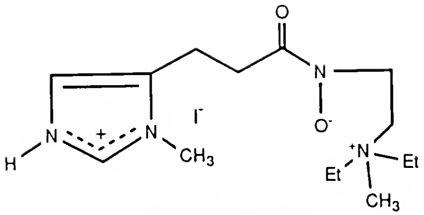
14



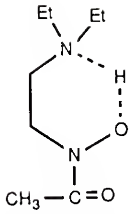
15

Repeated washings and trituration with solvents of the oily product yielded a pure sample of 14, which gave satisfactory C.H.N. analysis. However, the elemental analysis data for the bis dimethyl hydroxamic acid 15 varied over 1% of the expected values. The ^{1}H NMR spectrum of 15 showed four non-equivalent methyl peaks of which two equal intensity singlets at δ 3.0 and 2.90 correspond to the methyl group of the tetraalkylammonium moiety, and notably the spectrum showed two single peaks for each imidazole hydrogen. This is an indication for the existence of 15 in two different ionic (isomeric) forms.

It is logical to envision the existence of 15 partly in a zwitterionic form, where the anionic oxygen forms the counterionic interaction with the quaternary ammonium moiety through a 5-member ring. The low pK_a (~7.6) of the hydroxamic acids would allow part deprotonation of 15 in neutral medium. Intramolecular 5-member ring type interaction of anionic oxygen with the ammonium moiety has been explained by Bodor et al.[19] for the loss of reactivity (nucleophilicity) of some β-amino hydroxamic acids. As a consequence one would expect the reactivity of 15 to be different than that of 14. The physical chemical studies of these hydroxamic acids are cited elsewhere in this Chapter.



Zwitterionic 15



β-Amino hydroxamic acid

Synthesis of Oxime ECNR Compounds

The most logical synthetic scheme for oxime ECNR compounds was originally outlined (Figure 2-4) as preparation of 1-carboxymethyl pyridiniumaldoxime and subsequent tranesterification or esterification with an appropriate amino alcohol followed by quaternization of these esters with alkyl halides. The O-benzyl protected pyridinealdoximes were synthesized from the corresponding free aldoxime in 50-60% yield and were converted to 1-carbonylmethylpyridinium derivatives (71 to 73) by reacting with the appropriate haloacetate in solvents such as acetonitrile, ethanol and THF in a pressure bottle. However, under these conditions the reaction of bromoacetic acid with O-benzyl-2-aldoxime gave only 5% yield of O-benzyl-1-carboxymethylpyridinium-2-aldoxime bromide (71(b)), whereas O-benzylpyridine-3-aldoxime gave an 80% yield of the corresponding carboxymethylpyridinium acid (72). This explains the reduced nucleophilicity of O-benzylpyridine-2-aldoxime relative to the O-benzylpyridine-3-aldoxime as the result of increased delocalization through the 2-oxime moiety and the steric effect of the benzyl group on the ring heteroatom.

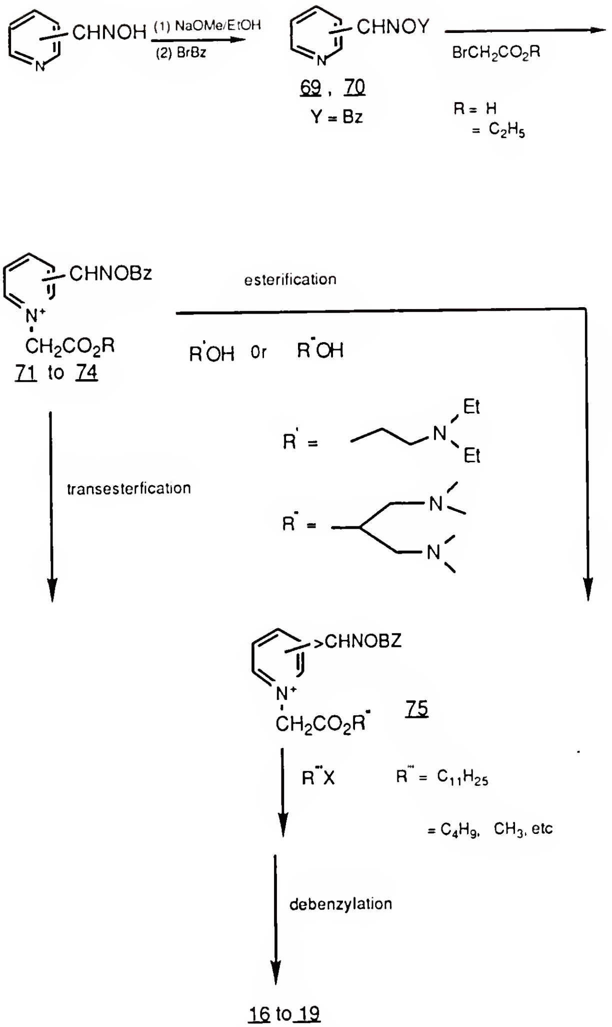


Figure 2-4: General synthetic schemes for oxime ECNR compounds.

Attempts at tranesterification of 1-ethoxy carbonylmethylpyridinium-O-benzyl-2-aldoxime (71(b)) with 1,3-bis(dimethylamine)-2-propanol or N,N-diethylethanolamine under various conditions ($ZnCl_2$ catalyst, p-toluene sulfonic acid etc), resulted in formation of a salt of 1-methyl-2-pyridiniumaldoxime (74) and decompostion products as identified by ^1H NMR. A probable mechanism is outlined in Figure 2-5. Katritzky and co-workers[61] have realized the formation of 1-methylpyridinium salts on heating t-butoxycarbonylmethyl pyridinum salts and postulated a decarboxylation pathway followed by the formation of 1-carboxymethylpyridinium betaine. In addition, the basicity of the amino alcohols would assist the formation of a betaine ester of(71(b)) by abstracting proton from the carboxymethyl moiety, which can lead to the decomposition of 71(b).

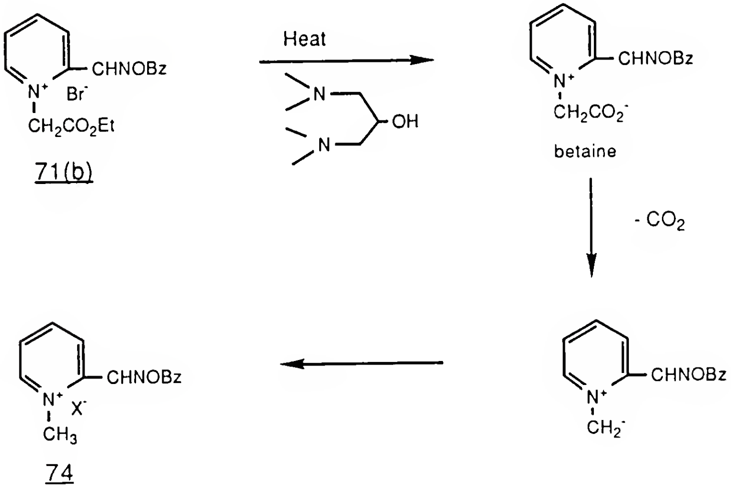
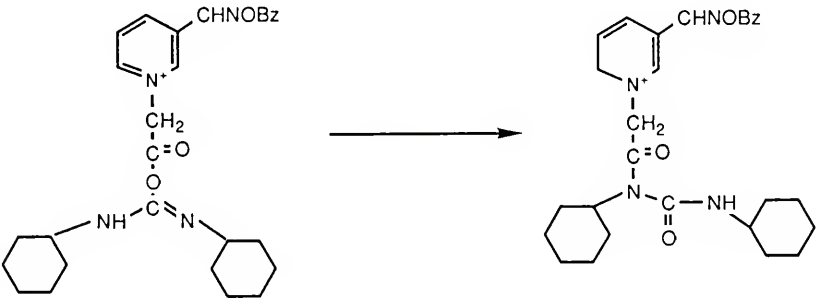


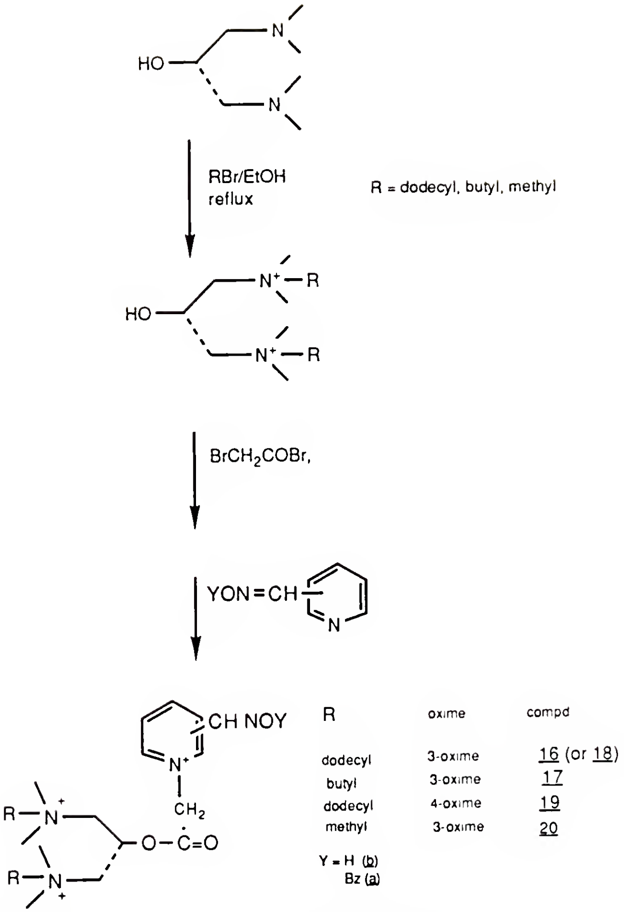
Figure 2-5: Proposed pathway of decarboxylation reactions of the 1-carboxymethylpyridinium-2-aldoxime

Alternatively, coupling of the aminoalcohol (1,3-bis(dimethylamino)-2-propanol or N,N-diethylethanolamine) to the pyridinium carboxylate moiety was attempted through esterification of O-benzyl-1-carboxymethylpyridiniumaldoximes (2- and 3-oximes). This reaction was attempted in the presence of the condensing agent dicyclohexylcarbodiimide (DCC) or diisopropylcarbodiimide (DIC) and a catalyst, like 4-dimethylaminopyridine (DMAP) or hydroxybenzotriazole (HOBT), these reactions lead to no trace of the expected product (75). Instead a dicyclohexylurea (DCU) adduct[62] of 72 was obtained. None of the attempts, using different molar ratios of DCC/DMAP or DCC/pyridine, and various conditions were found to carry the reaction beyond the formation of the initial DCU adduct. The stable adduct was isolated by column chromatography and found to have structure 76 on the basis of mass spectrum (M: 477) and ^1H NMR. Diisopropylcarbodiimide (DIC), often used[63] as the condensing agent in solid phase peptide synthesis, was found to have little or no reactivity with 1-carboxymethylpyridinium salts (1-pyridinium carboxylic acids).



Initial DCU adduct

N-Acyl urea (76)



R	oxime	compd
dodecyl	3-oxime	16 (or 18)
butyl	3-oxime	17
dodecyl	4-oxime	19
methyl	3-oxime	20

Y = H (b)
Bz (a)

Figure 2-6 : Alternative synthetic scheme to pyridinium ECNR: Via quaternization with bromoacylester of aminoalcohols.

Since all routes to 75 via esterification of carboxymethyl pyridinium salts were unsuccessful, a direct pathway (Figure 2-6) to target compounds which involved quarternization of pyridinealdoxime with appropriate bromoacylesters was attempted. The success of this scheme relied heavily on the preparation of the bromo ester of the amino alcohol, particularly the 1,3-bis(dimethylamino)-2-propanol.

The problem of self quaternization of free amino acylesters was avoided by using N-alkylated aminoalcohols (79(a)-(c)). Attempts to acylate 79(b) and 79(c) with bromoacetyl bromide in chloroform in the presence of a base such as pyridine, diisopropyl ethylamine and triethylamine gave a inseparable mixture containing the desired bromoester (^{1}H NMR), salt of the base and unreacted alcohol.

While literature procedures on bromo acylesters of aminoalcohols and their quaternized derivatives are scanty, a convenient way to 80-81 was discovered. A reaction of neat bromoacetyl bromide in the absence of any solvent or base, with 1,3-bis(alkyldimethyl)-2-propanol at 40°C afforded the desired bromoester in excellent yield (70-85%). The driving force for the reaction is the elimination of HBr gas. These bromoesters were characterized from ^{1}H NMR and mass spectral (FAB) data and used in the next synthesis without further purification.

In contrast, 1,3-bis(trimethylammonium)-2-propanol (79(a)) failed to give the corresponding bromoester (80(a)) by direct acylation methods. Attempts to prepare 80(a) using the activated ester method[63] and mixed anhydride method[64] as well as the reaction with bromoacetic acid anhydride were unsuccessful. The reduced reactivity for acylation appears be the result of insolubility of the quaternary alcohol 80(a) in aprotic solvents.

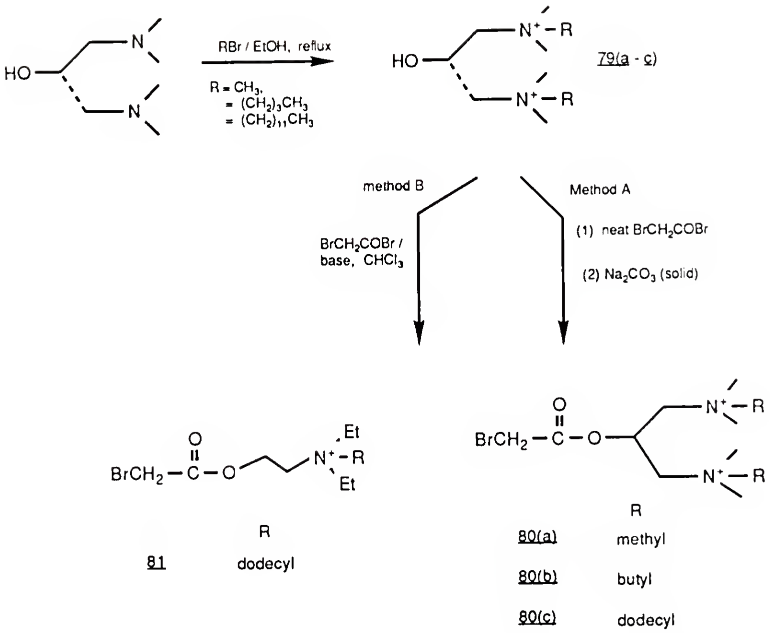


Figure 2.7 : Preparation of bromoacylester of quaternary aminoalcohols.

The final step in the preparation of oxime ECNR (16-19) involved quaternization of the bulky bromoesters (80(b), 80(c) and 81) with protected or unprotected pyridinealdoxime, which initially appeared impractical but proved to be a facile and general method of preparation of potentially diverse analogues of pyridinium-1-alkoxycarbonylmethyl compounds. The reaction of the bromo acylester of ammonium alcohols with pyridinealdoxime in 1:1 molar ratio in acetonitrile under positive argon pressure afforded the target ECNR compounds in 40-55% yield. It is noteworthy that unsatisfactory (<10%) yields are obtained with solvents such as DMF and chloroform, and the increase in the reaction temperature over 40°C resulted in partly decomposed products.

The ^{1}H NMR in DMSO-d_6 of all ECNRs (16-19) showed the expected pattern. A down field single peak (δ 12.9 to 12.35) for the OH proton and a single peak (~ δ 8.4) for the CH=N proton were seen for each ECNR, consistent with its existence in one isomeric form. On the basis of the OH down field signal (δ 12.9 to 12.35) the E configuration was assigned to 16(b), 18(b) and 19(b) by analogy with the literature result.[65] The ^{1}H NMR spectra in DMSO-d_6 of 16(b), 18(b) and 19(b) are given in Figures 2-7, 2-8 and 2-9. Unexpectedly these spectra showed deuterium exchange of the carbonylmethylene peak (~ δ 6.0) on addition of CD_3OD in all cases. The enol betaine behavior of the carbonylmethylene group is the result of neighboring pyridinium and the carbonyl groups.[61] However, pyridiniumaldoximes of simple acylesters such as 21 (1-octyloxycarbonylmethyl-3-hydroxyiminomethylpyridinium bromide) and 22 (1-dodecyloxycarbonylmethyl-3-hydroxyiminomethylpyridinium bromide) showed no deuterium exchange of carbonylmetylene protons. This suggest that the quaternary ammonium moiety must influence the acidity of the carbonylmethlene group in compounds 16 to 19.

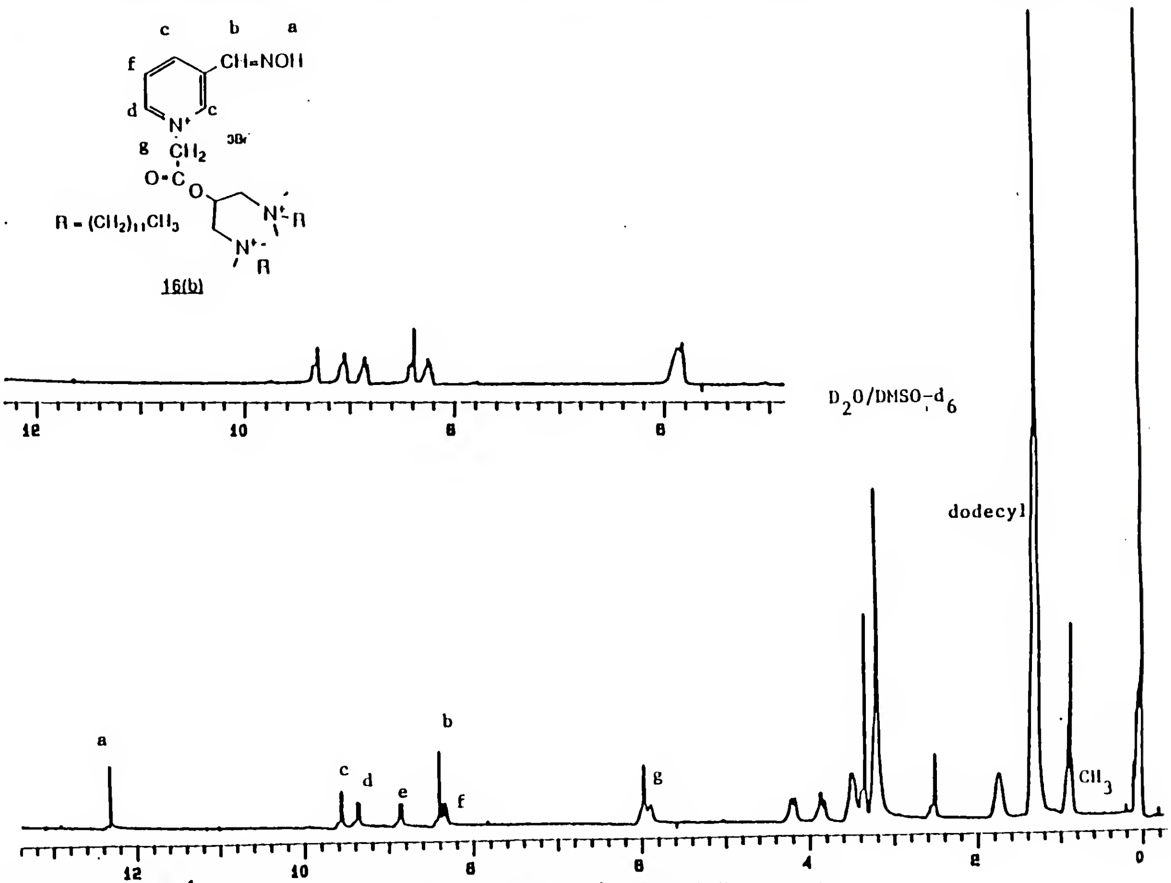


Figure 2-8: The ^{1}HNMR spectrum (300 MHz) of 16(b) in DMSO-d_6/trace H_2O.

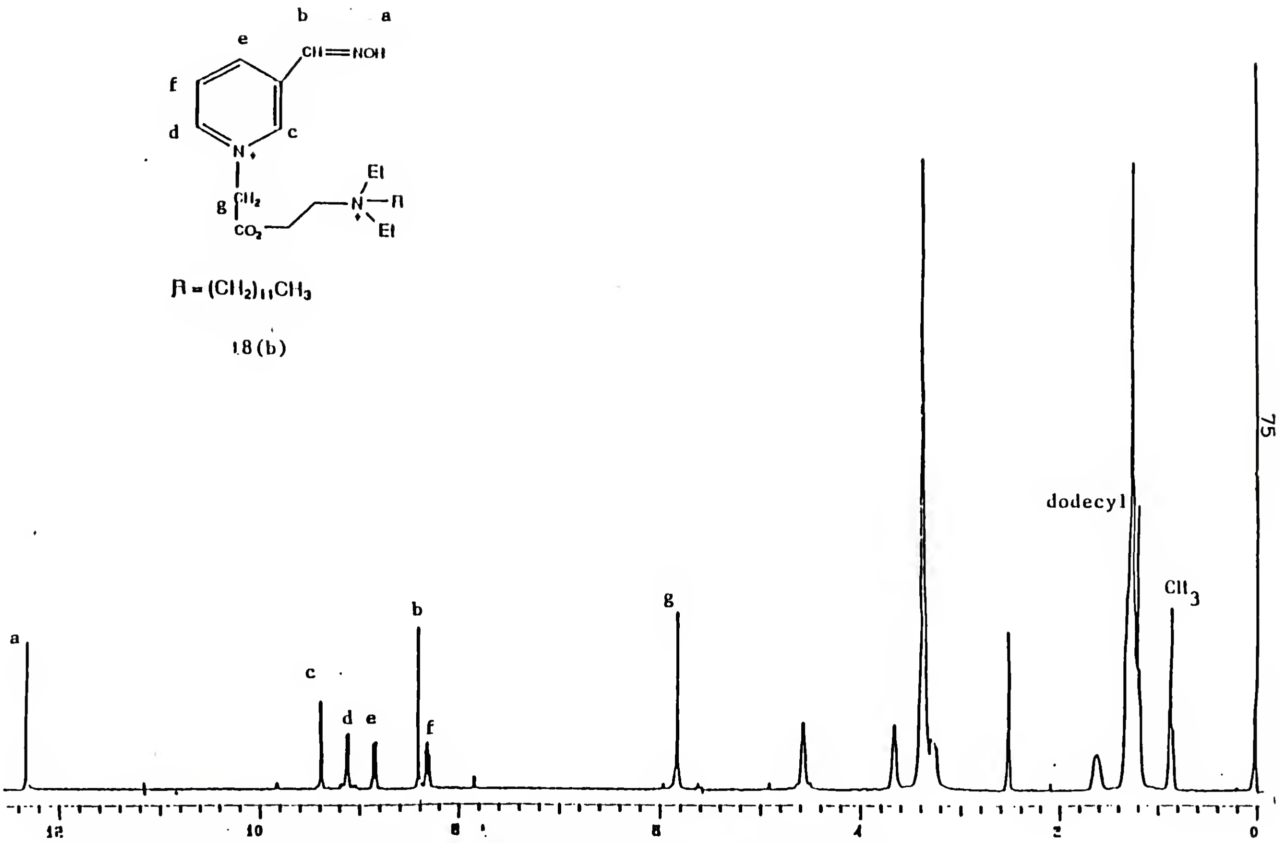


Figure 2-9: The ^{1}H NMR spectrum (300 MHz) of 18(b) in DMSO-d_6/trace H_2O.

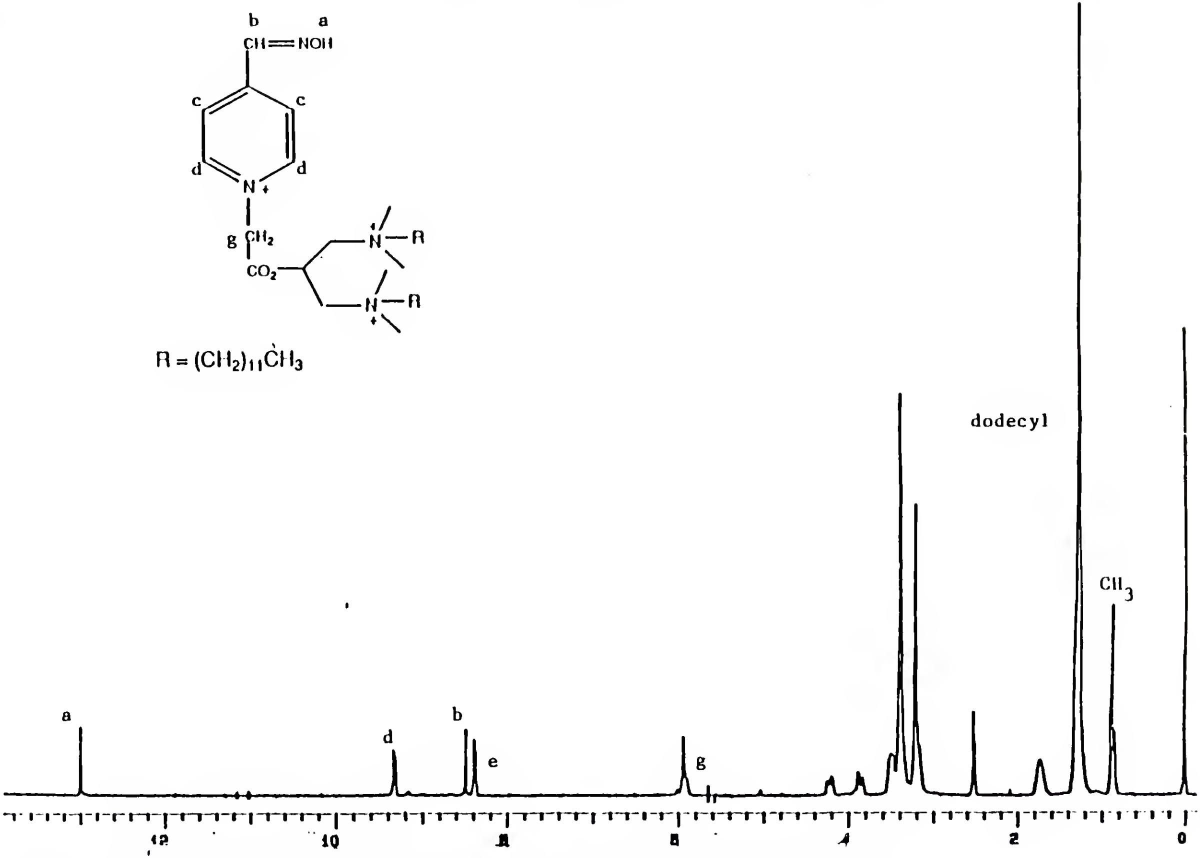


Figure 2-10: The ^{1}H NMR spectrum (300 MHz) of 19(b) in DMSO-d_6/trace H_2O.

Further characterization of compounds 16 and 22 were obtained from their elemental and mass spectral data. Some limitations[66] to the use of the FAB technique in gaining molecular weight information of the pyridinium salts with long chain tri and dicationic derivatives 16, 18 and 19: the mass spectra showed pattern for intense fragmentation. Attempts to convert the benzyloxime to the corresponding hydroxamic acid by hydrogenation over Pd-C gave products that lacked the carboxy ester portion. This indicates that selective removal of the benzyl group or hydrogenation of the imine bond can not be performed with the activated pyridinium ester systems like 16 to 22 without cleaving the ester bond.

Synthesis of Organophosphate Esters

The synthetic schemes for the synthesis of phosphorus esters that are used as substrates to evaluate the hydrolytic potential of ECNR are outlined in Figure 2-11. In general all phosphates were designed and synthesized to carry a hydrolytically labile chromophore, such as 4-nitrophenol or 2,4-dinitrophenol.

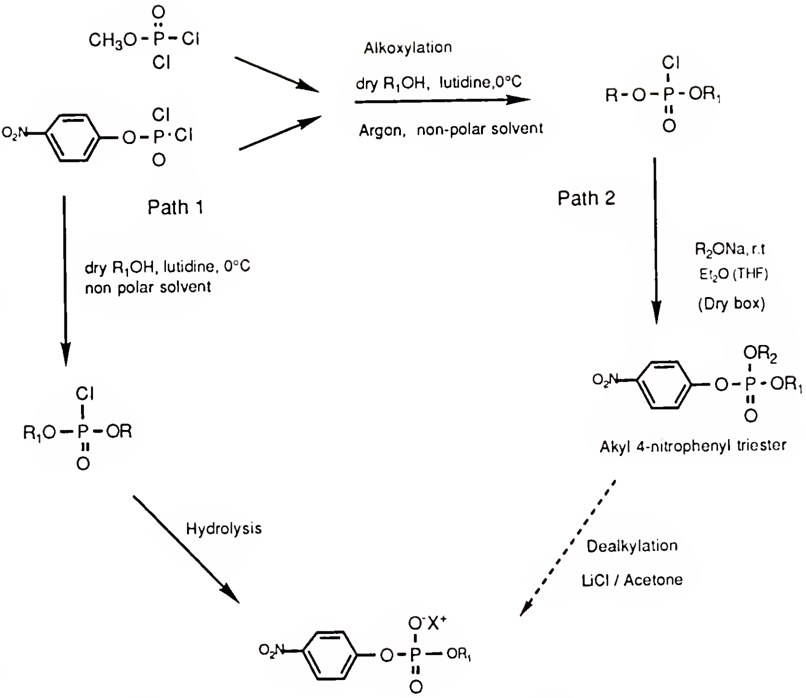
In general, organophosphate triesters that are analogues of nerve agents are not available through commercial sources, and therefore must be prepared in the laboratory prior to use. There are references cited in the literature for the synthesis of 4-nitrophenyl derivatives of phosphoric acid triester. However, no references were found for the synthesis of 4-nitrophenyl phosphoric acid esters carrying alkyl chains larger than the butyl group. According to a method (Figure 2-11, Path 2) described by Loew and Jeffrey[67] methyl dichlorophosphate and 4-nitrophenyl dichlorophosphate was sequentially alkoxylated to give mixed esters octyl methyl 4-nitrophenyl phosphate (23) and octyl bis(4-

nitrophenyl phosphate (24), respectively, in excellent yield. The use of non polar solvent (ether) and hindered base (2,6-lutidine) are reasoned to stop the reaction at the mono alkoxylation stage in the first step and to inhibit nucleophilic displacement of alkoxy groups in the second step. It is noteworthy that the insolubility problem in the second step of the reaction was avoided by carrying out the addition of alkoxide in portions in the dry box.

The triesters isolated were characterized by ^{1}H NMR, UV and mass spectra (EI), and showed only a trace amount of 4-nitrophenol impurity. Since this impurity does not affect the kinetics of the triester hydrolysis, it was decided to use these products for the hydrolytic study without further purification.

Owing to the highly toxic (cholinesterase inhibitor) nature of the products 22 and 23 very strict safety measures were assured for handling and disposing these compounds and their synthetic intermediates. All reaction residuals and glassware were carefully decontaminated with 2% sodium hydroxide solution.[28]

The organophosphate diesters are often prepared from phosphotriesters.[68,69] This method is routinely employed in the oligonucleotide synthesis which involves dealkylation (demethylation) and debenzylation of the triester. This conversion requires special reagents (generally strong nucleophile) such as magnesium-tetrahydrofuran complexes[70], thiophenol[69] and lithium chloride[11], and this approach is distinctive and indirect. Only the mono nitrophenyl phosphoric acid esters are prepared by direct phosphorylation of the corresponding nitrophenol (or its salt).[71,72]



Diester

R	R_1	
4-PNP	dodecyl	26
4-PNP	octyl	27
4-PNP	butyl	28
4-PNP	ethyl	29
4-PNP	benzyl	30
4-PNP	2,4-DNP	31
2,4-PNP	2,4-DNP	33

Triester

R_1	R_2	R_3	
methyl	octyl	4-PNPP	23
octyl	4-PNP	4-PNP	24

X = lutidine, H, Na, Li, etc.

Figure 2-11: General synthetic sequence for esters of 4-nitrophenyl phosphoric acids.

Despite the potential use of 4-nitrophenyl phosphate diesters in hydrolytic studies of phosphate diesterase enzymes, it is surprising that there are only a few examples[68] in the literature for the direct preparation of these esters. In general, application of the triester method to synthesize diester monoanion suffers from three major setbacks: First, this method involves two or three alkoxylation steps from the starting phosphorylating agent and in some cases poor yield[67] due to low percentage yields in the first alkoxylation step. Second, most all 4-nitrophenyl triesters of phosphoric acids (e.g., Paraoxon) are highly toxic nerve agents and, therefore, cause problems in the handling and disposal of the compounds. Third, dealkylation (usually demethylation) or debenzylation would not be selective with mixed triesters carrying other labile groups and in some cases would require use of moisture sensitive reagents.

Turner and Khorana[68] have described phosphorylation of thymidine-3' to its triester and diester with 4-nitrophenyl phosphodichloridate (82). However, it appears that direct use of 82 to make long chain or other mixed 4-nitrophenyl diesters and their salts has not been explored so far. The direct reaction of 82 with alcohols of interest under conditions employed for triester synthesis gave the corresponding monochloridate, which then on differential treatment either with lutidine or acidic water gave the corresponding diester phosphate. The 4-nitrophenyl monochloridate was readily hydrolyzed with aqueous 2,6-lutidine to provide the lutidine salt in 50-60% yield. The isolated salt was found to be >99% pure by ^{1}H NMR and UV. Increased solubility of the lutidine salts of octyl and dodecyl derivative (26 and 27) in organic solvents increased their yield slightly over butyl and benzyl (28 and 29) counterparts. An attempt to alkoxylate 82 with 2,4-dinitrophenol resulted in the isolation of the crude lutidine salt of 31, which was evident from the ^1H NMR, but could not be purified.

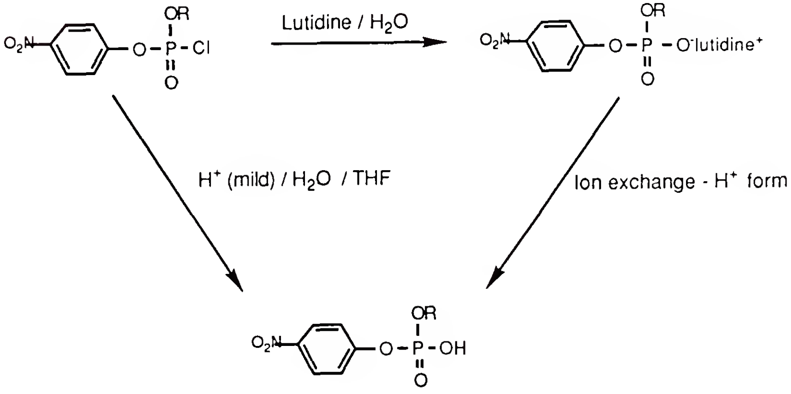


Figure 2-12: Diester hydrogen phosphate synthesis.

The neutral hydrogen phosphate form of the diesters were obtained either by eluting the lutidine salt through the acidic Amberlyst ion exchange column or directly from the monochloridate by hydrolyzing in mildly acidic water-THF followed by extraction with an organic solvent (chloroform). In contrast to the lutidine salt, the neutral form of the diester is generally a solid, which was purified by recrystallization in mixed solvents to give pure hydrogen phosphate diesters (26 to 30). The low yield obtained for the ethyl 4-nitrophenyl phosphate (30) is the result of its increased aqueous solubility. All compounds were found to be hygroscopic, and particularly, the dodecyl (26) and the octyl (27) derivatives showed decomposition at 25 °C.

Overall, the direct method employed here for the synthesis of 4-nitrophenyl phosphate diesters afforded a versatile, convenient and non hazardous synthetic route to spectroscopically pure diesters. The mass spectra (EI) gave the molecular ion (M^+) in all cases except the benzyl derivative 30 (M-1 peak). The C,H,N results were within accepted values and in most cases accounted for the hydrated forms. The UV spectra of diesters (26-32) showed λ_{max} around 294 nm and in some cases absorption maxima were seen at two wavelengths (274 and 294 nm).

The ^1H NMR spectra of compounds 26 to 30 agreed well with the assigned structures. Figure 2-13 shows a ^1H NMR spectrum of n-butyl 4-nitrophenyl hydrogen phosphate. The ^{31}P - ^1H coupling caused a distinctive double split of P-O-methylene group with a coupling constant of J≈ 14 Hz.[67] The chemical shift of P-O-methylene hydrogens showed a up field shift (δ 4.40 to 4.05) on conversion of the phosphomonochloridate to the hydrogen phosphate (or lutidine salt). The ^{13}C NMR spectra of these diesters showed distinctive $^{31}P-^{13}C$ coupling of P-O-C carbons.

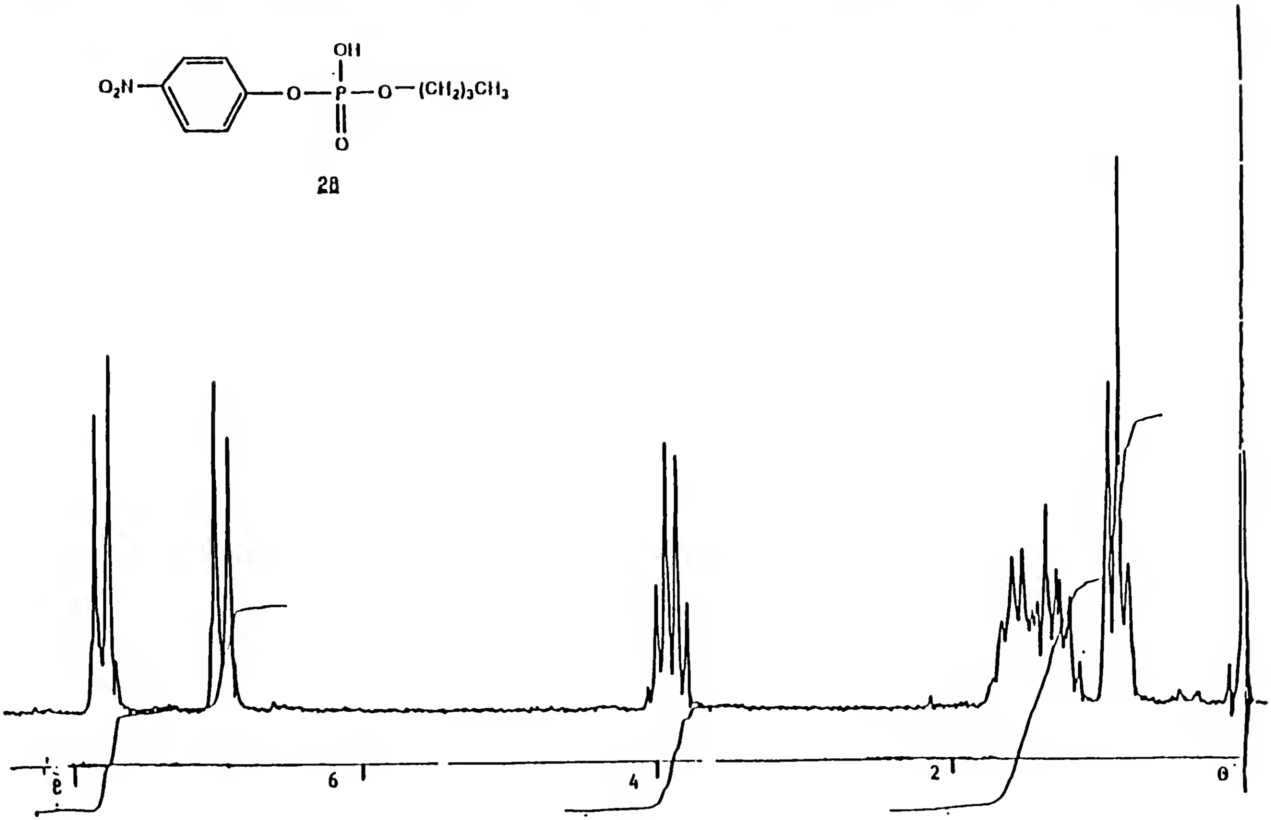


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Figure 2-13: The ^{1}H NMR spectrum (90 MHz) of n-butyl 4-nitrophenyl hydrogen phosphate in $CDCl_3$.

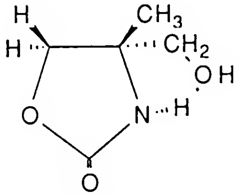
Several attempts to synthesize bis(2,4-dinitrophenyl) phosphate (33) using a method described by Vilkas and coworkers[73] were unsuccessful. The product isolated upon reaction of phosphorous oxychloride with excess of 2,4-dinitrophenol in 2,6-lutidine gave a salt that accounted for only one nitrophenyl group (^{1}H NMR) and less or no 33. This salt turned pink on treatment with alkali. Bunton and Farber[74] suggested this to be due to the formation of an opened chain derivative of the dinitrophenyl group. Similar results were encountered when pyridine was used as the solvent/base. It seemed that the major problem was in the insolubility of the 2,4-dinitrophenol in the reaction medium (pyridine or 2,6-lutidine). Therefore, a modified reaction condition, a homogeneous solution of 2,4-dinitrophenol (recrystallized) in acetonitrile/2,6-lutidine was reacted with redistilled phosphorous oxychloride for a short period which on hydrolysis provided the lutidine salt of 33 in modest yield (40%). The melting point of the recrystallized product agreed with the literature value.[11]

Synthesis of N-Bromo Compounds and their Derivatives

Oxazolidinone Systems

The 2-oxazolidinone ring is often used as a protective system for β-hydroxyamino groups. Some 2-oxazolidinones are physiologically active[75(a)] and have a number of industrial uses. Some have uses in optical resolution processes of active β-adrenergic receptor blocking agents (e.g., metoprolol[75(b)]).

Preparation of 2-oxazolidinone usually involves reaction of the appropriate amino alcohol with a carbonyl synthon such as diethyl carbonate[76a] (Et_2CO_3), acetone, carbonyl imidazole[76] or urea[76b,77], or can be obtained from the decomposition of β-hydroxyethyl urea[77]. Bodor et al.[44] accomplished synthesis of 4,4-dimethyl-2-oxazolidinone (42) compounds by reacting 2-amino-2-methyl-1-propanol with anhydrous ethyl carbonate in the presence of sodium ethoxide as the catalyst.[44] In view of the expected interaction of the second alcohol group of 2-amino-2-methylpropane-1,3-diol (83) with anhydrous ethyl carbonate, therefore, 4-hydroxymethyl-4-methyl-2-oxazolidinone (44) was prepared by a method described by Horn et al.[77] This involved thermal decomposition of substituted ß-hydroxyethylurea, which was formed in situ by the reaction of 83 with urea in DMF at high temperature (150°C) to provide 44 in 60% yield. Liberation of ammonia gas was envisioned to be the driving force in the two consequtive reactions taking place in the same vessel without interruption. The product obtained agreed with all physical constants reported for 44. The ^{1}H NMR spectrum showed AB coupling (J_{AB} = 9.5 Hz) for the oxazolidinone ring methylene hydrogen. Compound 44 can be envisaged to exist in a form in which the more acidic amide hydrogen is intramolecularly hydrogen bonded to the oxygen atom of the hydroxyl group.



44

Sulfation of 44

Functionalization (Figure 2-14) of the hydroxyl group of 44 with solubilizing groups is made taking advantage of the hard basic character of the hydroxyl group. A sulfated hydroxyl group was the major target because of the simplicity of the reaction and the highly polar nature of the sulfate group. The sulfation of primary alcohols is often carried out with an electrophilic sulfur trioxide-base complex and the preparative methods used were generally developed for the synthesis of sulfate nucleosides.[78] The sulfation of 44 was carried out by reacting with sulfur trioxide-pyridine complex in warm acetonitrile to give the exclusively O-sulfated product 46(a) in 60% yield. Compound 46(a) was characterized on the basis of its ^{1}H NMR spectrum 46(a), which showed a distinctive down field shift (δ 3.45 to 3.90) for the methylenesulfate hydrogens and a broad peak at δ 6.7-6.5 for the free amide hydrogen proton (N-H). Further characterization was obtained from the mass spectral (EI) and C,H,N analytical data. The sodium salt of 46(a), was obtained by treatment with aqueous sodium hydroxide, and after selective removal of inorganic salts provided a hygroscopic sulfate salt (47(b)) in 42% yield. It should be noted that all sulfate derivatives exhibited increased aqueous solubility compared to the their hydroxyl precursor 44.

Prior to the discovery of the above mentioned route (Figure 2-14, Path B) the preparation the O-sulfated salt of 44 was originally planned through a route (Path A) that involves protection of the amide nitrogen. This was suggested in view of the fact that 44 could be sulfated both at the amide group and hydroxyl group. The N-protection was accomplished by reaction of a benzyl carbamate[79], which involved reaction of equal molar sodium carbonate and benzochloroformate in aqueous medium to provide 70% yield of 84. The sulfation of 84, and the following deprotection of the protective group by catalytic hydrogenation to provide 46(a) was not attempted because Path B was proven to be a short and simple route to compound 46(a).

Initially, N-bromination of 44 and 46(b) were carried out with bromine - aqueous sodium hydroxide.[45] The N-brominated 44 (45) was isolated on precipitation from the reaction mixture whereas the N-bromo sulfate derivative (47) was isolated from the aqueous layer on freeze-drying. However, this brominating method unavoidably produced an inseparable sodium bromide - 47 mixture. All attempts to remove sodium bromide with solvents such as n-butanol caused further decomposition of the N-bromo product. In a modified reaction, use of bromine exchange reaction of N-bromosuccinimide (NBS) with 46(b) in a carbon tetrachloride-water mixture was attempted. The solids obtained from the exchange reaction contained only 70% 47, based on molar bromine content, and the product contained succinimide as the major impurity. In order to prevent contamination of inseparable by-products a relatively uncommon brominating agent, t-butyl hypobromite (b.p 115-120°C)[80], was used to provide a complete conversion of 46(b) to 47. The aqueous phase reaction (bromine exchange) of t-butyl hypobromite parallels that of NBS. The preparation t-butyl hypobromite involved first preparation of aqueous hypobromous acid (HOBr) in situ, which was achieved by reacting silver sulfate with aqueous bromine[80] (Figure 2-15). The reaction of hypobromous acid with t-butanol gave t-butyl hypobromite in a crude yield of 70-80%. The reaction of t-butyl hypobromite with aqueous solution of 46(b) yielded 47 with practically quantitative (100%) bromine content. The advantage over other brominating agents is that t-butyl hypobromite yields t-butanol on reaction, which is easily removed by evaporation. t-BuOBr is a strong brominating agent which does not require an inorganic base (NaOH) for the bromine exchange reaction.

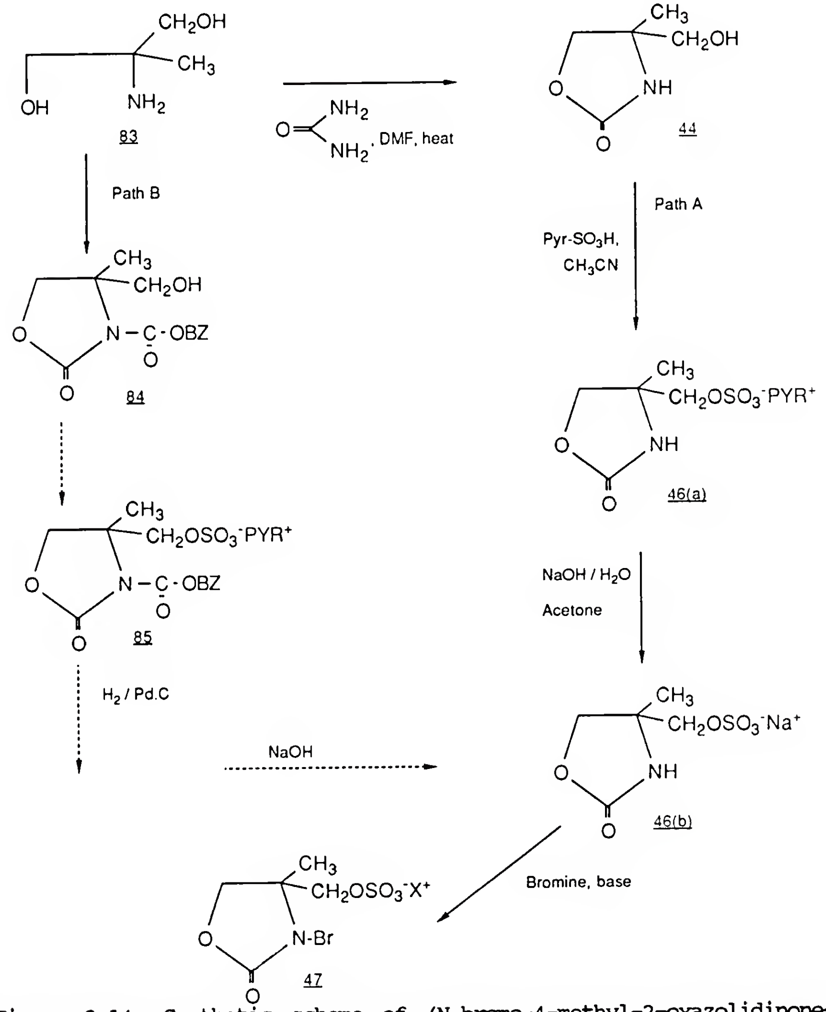


Figure 2-14: Synthetic scheme of (N-bromo-4-methyl-2-oxazolidinone-4-yl)methyl sulfate.

The product 47 showed a ^{1}H NMR spectrum only slightly differnce from that of 46. The mass spectra of 47 with FAB (or EI) did not provide molecular ion information due to the intense fragmentation of the molecule. The iodometric titration of 47 showed one equivalent of bromine incorporation. The UV spectrum showed $_{max}$ (pH 9.3) at 273 nm, which is in close proximity with the absorption wavelength of 3-bromo-4,4-dimethyl-2-oxazolidinone ($_{max}$ 278 nm).

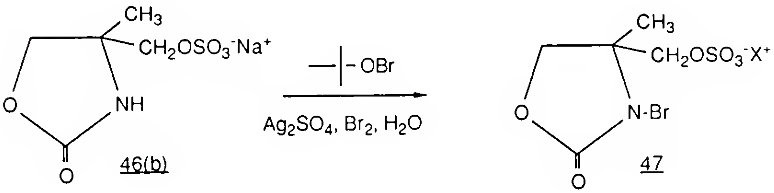


Figure 2-15: Bromination of (4-methyl-2-oxazolidinone-4-yl)methyl sulfate

Succinoyl Derivative of 44

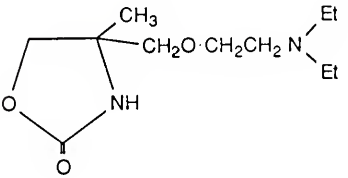
The synthesis of 4-succinyloxymethyl-4-methyl-2-oxazolidinone (48) was accomplished by reacting 44 in the presence of equal molar pyridine and a slight excess of succinic anhydride to obtain 49 in 73% yield. It should be mentioned that the use of excess pyridine (more than 1.05 equivalent) resulted in a mixture of products, which may well be due to competing acylation at the amide nitrogen.

The ^{1}H NMR of 48 in $CDCl_3$ solution indicated a singlet (δ 2.5) for the succinoyl methylene hydrogens, and a down field peak at δ 4.10 was observed for methyleneoxy hydrogens which coalesced with the AA'BB' quartet of the oxazolidinone ring protons. The mass spectrum (EI) of 48 showed an intense peak (M$^+$) at the expected value for the free acid.

The N-bromination of 48 was carried out with aqueous bromine-sodium hydroxide (2NaOH/Br$_2$), a method described for N-bromination of 4,4-dimethyl-2-oxazolidinone (42)[45] and yielded the N-brominated carboxylate salt of 48 (49) was obtained in 36% yield. The characteriztion of 49 by the iodometric tiration showed >95% positive bromine content. The low yield may well be the result of a side reaction, the oxidation of the carboxylate moiety of 48. The mass spectrum (EI) of 49 clearly showed intense isotope peaks of the fragmentation ion (M-CH$_2$CO$_2^-$) for the loss of acetate group from the molecular anion at the expected values of 253 (^{82}Br) and 251, (^{80}Br) which is consistent with the structure.

Diethylaminoethoxy derivative of 44

The insertion of β-diethylaminoethanol moiety to 44 was aimed as a strategy by which retain a highly polar quaternary ammonium group can be introduced to the molecule. The reaction of chloroethyldiethylamine with bis anionic 44 gave the expected O-alkylated product 50.



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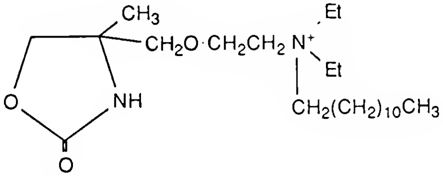

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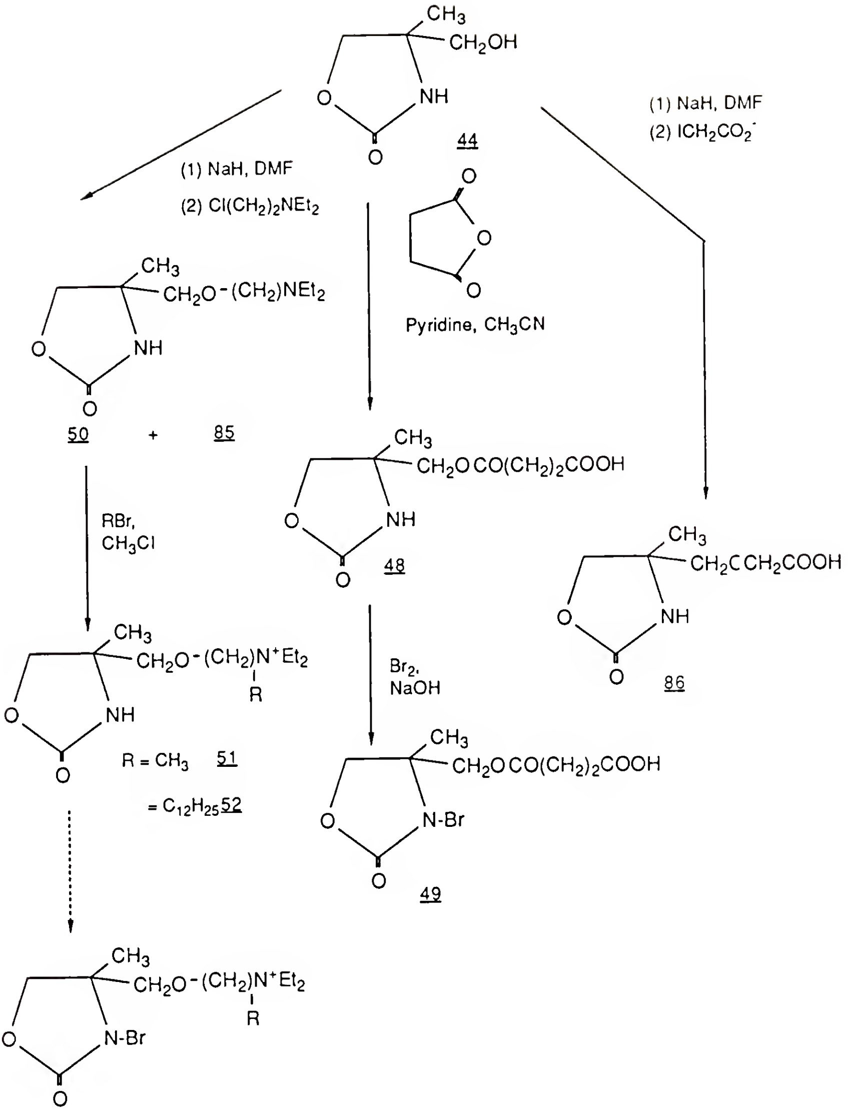
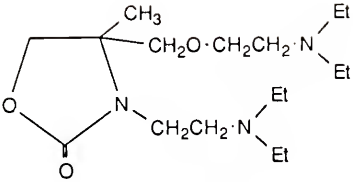


Figure 2-16: Synthetic scheme for polar derivative of 4-hydroxymethyl-4-methyl-2-oxazolidinone.

The reaction involved generation of dianionic 44 in situ with two molar equivalent of sodium hydride, in which the alkoxy anion is more basic than the amide anion, and therefore, was expected to react first. The product mixture obtained contained predominantly (9:2) the O-alkylated 85. Separation through a silica gel column afforded O-alkylated product (50) in 33% yield and a less polar component (oil) was obtained in 9.3% yield. The ^{1}H NMR of 50 showed a distinct broad peak at around δ 7.5, which appears in the region for the acidic hydrogen of the amide hydrogen. The IR spectrum (NaBr) of 50 showed a marked decrease in the broadness at 3500-3100 cm^{-1} contrast to the parent molecule 44, which is in good agreement with the loss of the hydroxyl group. However, more confirmative evidence for O-alkylation was obtained from the ^{13}C NMR (decoupled and APT) study (Figure 2-17) of 44, 50, and 1,3-bis(dimethylamino)-1-propanol (83) as well as other model compounds such as propanol, propyl ether and propoxyethanol.

The ^{13}C chemical shift of the hydroxymethylene carbon (CH_2OH) of 44 shows a significant downfield shift by about 5 ppm on alkylation of the oxygen.[57] In addition, N-alkylation of 44 should significantly alter the chemical shift of the neighbouring (carbonyl) carbon (C-2), but 50 appears to have almost identical chemical shift (δ 158.7 and δ 159.0) that of its parent molecule 44. These data are in close agreement with the O-monoalkylated product (50). A trial bromination of 50 yielded a brominated product having one equivalent of bromine incorporated (iodometry). On the basis of the mass spectrum, TLC and ^{1}H NMR the minor component (less polar component) isolated from the reaction mixture was assigned structure 85.



85

Quaternization of 50 with methyl iodide provided the water soluble N-diethylmethylammonium salt (51) in 76% yield. In a similar manner 50 was reacted with dodecyl bromide to the ammonium salt 52 (70% yield), which was found to be soluble both in water and in chloroform and, was expected to have micellar properties. The mass spectra (FAB) of 51 and 52 contained strong peaks (M^+) at the expected values of 245 and 399 respectively, which were consistent with their structure.

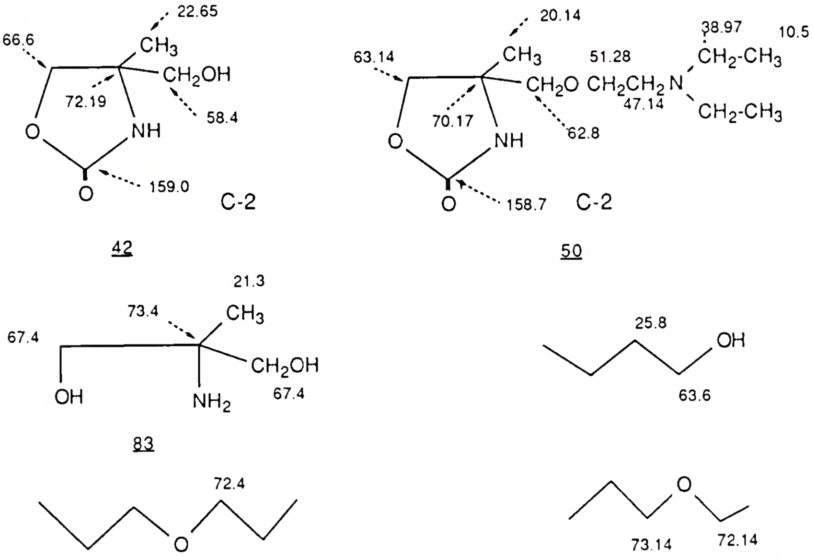


Figure 2-17: The ^{13}C chemical shift assignment of 4-(β-dimetylamino) ethoxymethyl-4-metyl-2-oxazolidinone (50) and model compounds.

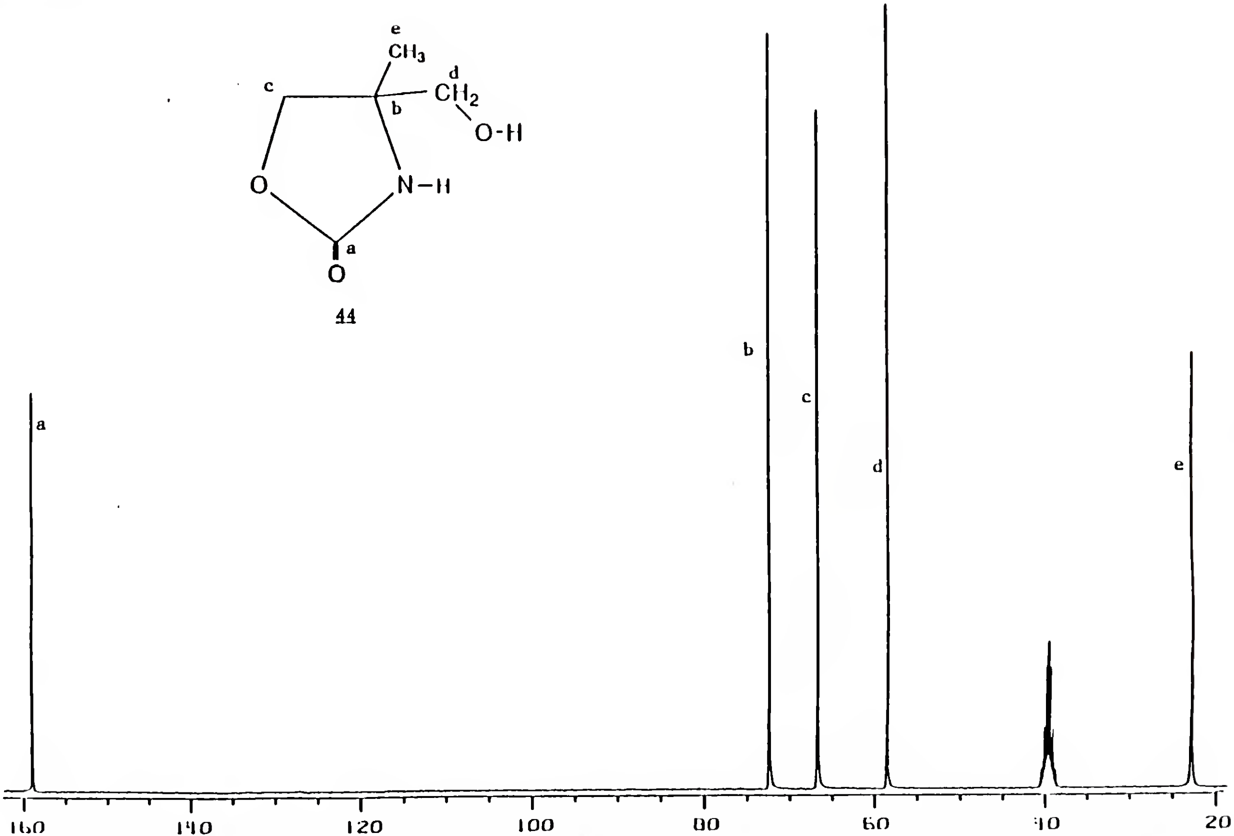


Figure 2-18: The ^{13}C NMR spectrum (74.5 MHz, DMSO-d_6) of 4-hydroxymethyl-4-methyl-2-oxazolidinone (44).

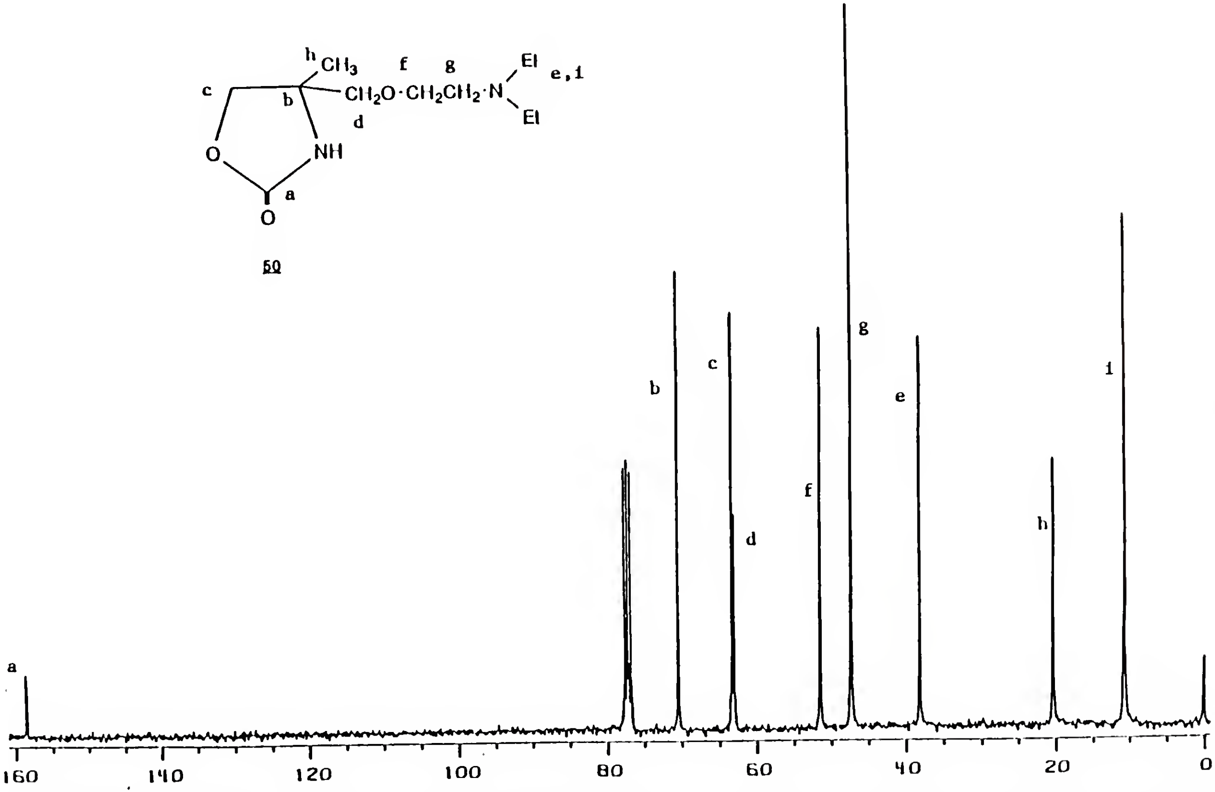


Figure 2-19: The ^{13}C NMR spectrum (74.5 MHz, $CDCl_3$) of 4-(β-dimethylamino)ethoxymethyl-4-methyl-2-oxazolidinone (50).

Dimethylglycouril System

Dimethylglycouril (54) was prepared according to the method described by Franchimont and Klobbie[50] using a condensation reaction of urea and methyl diketone. The compound 54(a) was found to be practically insoluble in all solvents including dimethyl sulfoxide and only soluble in trifluroacetic acid. Purification was achived by suspending it in hot dimethyl sulfoxide solution. The identity of compound 54(a) was established from the ^{1}H NMR spectrum in trifluoro acetic acid, which showed a singlet at δ 1.3 for methyl groups and a broad peak at δ 7.2-7.0 for the NH protons. The mass spectrum (EI) of 54(a) showed strong (M+1) peak at the expected value of 171.

The N-bromination of 54(a) was achieved with four molar equivalent of aqueous bromine sodium hydroxide, which provided the tetrabromo dimethylglycouril (54(b)) in 59% yield as a faint yellow non hygroscopic product. Iodometric titration of the product verified to have four molar bromine incorporation and the mass spectral (EI) analysis showed low intensity isotope peaks (M-6) at 486, 484 and 482 at the expected values. The ^{1}H NMR spectrum 54(b) was obtained in CD_3OD solution (sparingly soluble) immediately on preparation and showed slight down field (δ 1.3 to 1.5) shift for methyl protons from that of the parent molecule (54(a)). The product 54(b) was surprisingly stable relative to any N-bromo oxazolidinone drivatives, and found to have a half life over two years at room temperature.

An approach to monohydroxymethylate 54(a) was using literature methods [82,83] described for the monohydroxymethylation of 5,5-diphenylhydantoin. This method gave polyhydroxymethylated compounds under various conditions. Since compound 54(a) is a symmetric molecule, it exhibits no selectivity for monosubstitution. In addition, the subsequent reaction of formaldehyde with hydroxymethylated 54(a) appears to be drastically faster than the initial reaction due to the increased solubility of the hydroxymethylated products and hence leads to polysubstitution.

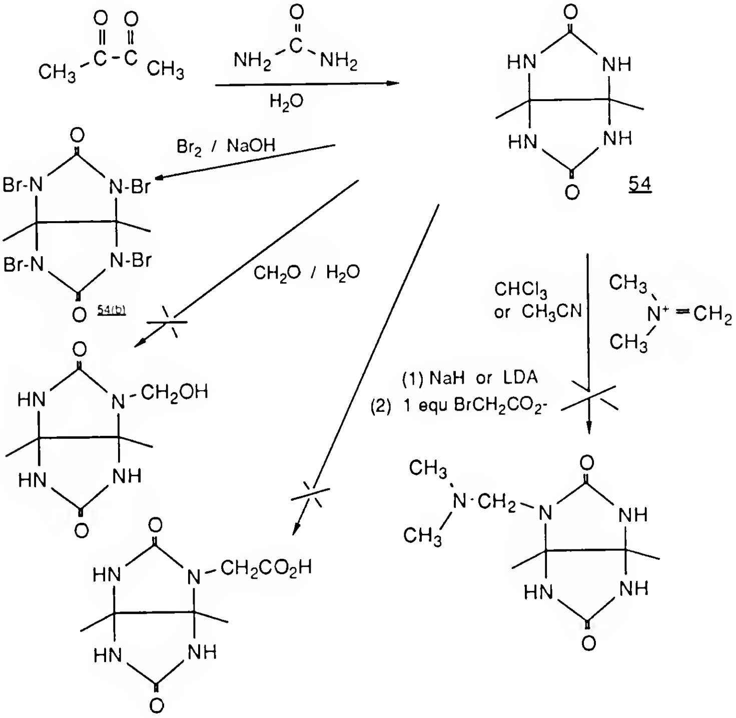
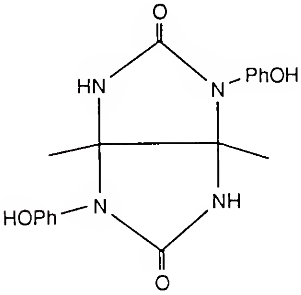


Figure 2-20: Synthetic scheme to dimethylglycouril (54) and attempted monosubstitution reactions.

Monofunctionalization was also attempted by reacting ethyl bromoacetate, succinic anhydride and Mannich bases with neutral and monoanionic (in situ) 54(a). All these attempts failed to give the desired product, but in most cases gave unreacted starting amide. It can be envisaged that the major problem is the insolubility of 54(a), practically in all protic and aprotic solvents. An alternative approach, through a retrosynthetic route, which involved condensation[84] of hydroxyphenylurea (86) with methyl diketone in the presence of TFA gave condensed product. This product appeared to have structure (^{1}H NMR) 88, but not the desired product 87.



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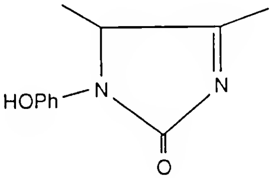

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Physical-Chemical Studies of ECNR Compounds

Determination of Physical Constants

Ionization constants

It has been established that the pK_a constitutes an important factor in the in vivo and in vitro efficacy of nucleophilic antidotes in reactivating inhibited enzyme.[1] Of the nucleophiles studied[1,23] so far, it is believed that for a maximum reactivation efficacy the reagents should fall within the pK_a values of 7.5 to 8.5, and anything outside of this range expected to show reduced reactivation of the inhibited enzyme. This emerges from the fact that an efficient reactivator should have adequate anionic concentration at the physiological pH, which indicates that the pK_a should be low enough to provide such concentration. On the other hand, too low pK_a values make the compounds too acidic and renders them less nucleophilic.

The pK_a of ECNR compounds 14, 16(b), 18(b) and 18(b) were determined spectrophotometrically. The analytical wavelength used was that at which the greatest difference between the absorbances of neutral and anionic species was observed. The average pK_a values that were determined from the sigmoid plots of absorbance against pH of the buffer solution around the half neutralization point using equation[15] 2-1.

$$pK_a = pH + \log \frac{[A_{max} - A_{obs}]}{[A_{obs} - A_{min}]}$$

Eqn 2-1

Table 2-1: Apparent ionization constants of ECNR and reference compounds.

Code names	Compd	pK_a [a]
HA	14	7.58 ± 0.03[b]
LlmHA[18]	5	9.04
ImHA[55]	88	9.50
Bis-4-oxime	19(b)	7.69 ± 0.01
Bis-3-oxime	16(b)	9.30 ± 0.08
3-PDI[15]	3(a)	9.38
Mono-3-oxime	18(a)	9.11 ± 0.03
2-PAM[1]	3	7.68

a: determined specctrophotometrically at 35 °C at in 0.05M buffer.
b: 0.03M buffer.

The apparent pK_a of 3-oxime ECNR 16(b) and 18(b) (9.30 and 9.11) can be compared with that of 1-alkylpyridinium-3-oximes (9.1-9.5)[15]. The apparent pK_a of 4-oxime ECNR 19(b) (7.51) can be compared with that of 1-methylpyridinium-4-aldoxime and 1-alkyl dimethylaminoethylpyridinium-4-oxime (8.25 and 8.40)[15]. It is apparent that the low pK_a value of the 4-oxime (19(b)) is the result of greater degree of delocalization in the oximate anion relative to the 3-oximes.

The increased acidity of 19(b) relative to the 1-alkylpyridinium-4-aldoxime analogues may well be due to the medium effect of the dicationic ammonium moieties.[86]

The ^{1}H NMR spectra of the oxime ECNRs (16 to 19) were indicative (deuterium exchange) of the acidic cabonylmethyl group in each case, which is can be predicted to have an acidity constant between diethyl malonate (pK_a 12.8)[86] and ethyl nitroacetate (pK_a 5.8)[86]. Unfortunately, in the spectrophotometric method it is more complex to determine pK_a values of two ionizable groups that lie within 3pK_a units of one another without the use of a computer program.[87,88] On the basis of spectral properties and pK_a values of reference compounds[15,85] the acidity can be assigned to ionization of the oxime proton.

The apparent pK_a (7.58) determined for the hydroxamic acid 14 is 1.0 to 2.0 units lower than the reference compounds 5 (9.38)[15] and N-laurylbenzohydroxamic acid (8.41)[18]. The pK_a for the deprotonation protonation at the basic nitrogen of 1,4-dialkyl substituted imidazoles has been reported to have values between 7.6-8.6.[89] However, this acid-base equilibrium is not expected to display significantly different UV-VIS spectra. This suggested that the pK_a observed for 14 involved the hydroxamate anion rather than the basic imidazole nitrogen. Tabushi et al.[89] have suggested that the micellar state may be involved in reducing the pK_a by assisting deprotonation of the hydroxamic acid.

Critical micelle concentration (CMC)

The CMC of micelles can be experimentally determined by several methods available that are summarized by Shinoda, et al.[90(a)] Fluorescence spectroscopy was employed to determine the CMC of those ECNR compounds that were expected to show micellar properties. Dissolution of the reagent in aqueous solution does not affect the fluorescence characteristics of a micellar reagent solution until the CMC is reached. Thus, an emission spectrum of a micelle just above the CMC shows a shift (variation) in its fluorescence character (e.g., λ_{max}) whereby changing the λ_{max} to another wavelength causes a conspicuous change in the fluorescence rate at the wavelength it was initially observed. These spectral shifts were used to determine the CMC of detergents that are difficult to determine by more conventional methods.[90,29]

Table 2-2: Criticle micelle concentration of some ECNR and reference compounds at 35°.

Code name	compound	10^3Xcmc, M	
HA	14[a]	2.28	(2.10)
L1mHA	5[b]	1.10[18]	
3-PDI	3(a)[c]	0.63[15]	
Bis-3-oxime	16(a)[d]	5.15	(4.65)
CTAB[e]		2.5[15]	

( ) at25°
a: buffer(0.03M), pH 9.3 (ext. 344: emis. 422 nm)
b: buffer(0.05M), pH 10.40 (surface method)
c: buffer(0.05M), pH 9.3 (ext. 410: emis. 478 nm)
d: buffer(0.05M), pH 9.3 (ext. 400: emis. 462 nm)
e: buffer(0.05M), pH 9.3 (surface tensio method)

The CMC values determined for 14 and 16(b) from the points of inflexion of these curves are 2.10×10^{-3} and 4.65×10^{-3} respectively at 35°C. Both (14 and 16(b)) showed a decrease in emission rate above their CMC. However, 16(b) shows a regain in fluorescence rate well above the CMC which may be an indication of a biphasic shift in the micelle monomer equilibrium[90(b)] state before and after the CMC. The CMC values of 14 (HA) and 16(b) (bis-3-oximes) is compared with those of some analogous compounds, 5[18] and 3(a)[15] in Table 3-2. On comparison, 16(b) shows higher (4.65×10^{-4} M) CMC value than its 1-alkyl derivative 3(a) (1.63×10^{-3} M), which may well be due to the increased polarity of 16(b) caused by the dicationic diammonium moiety. On the other hand, 14 exhibited a CMC value (2.28×10^{-3} M) close to that of CTAB (2.5×10^{-3} M) under similar conditions. The connection between the observed CMC and the reactivities of these ECNRs against organophosphorus compounds will be discussed in the next subheading.

Reaction Kinetics

General scheme

All kinetic experiments followed the general outline, given by equation 2-2, where K_{obs} is the observed pseudo first order rate constant at > 10 fold excess of nucleophile concentration over the fixed substrate concentration and K_2 is the apparent second order rate constant for the nucleophilic reaction, and K_b is the second order reaction for the buffer hydrolysis of the substrate. The K_{obs} is also equal to $K_{micelle}$ of the micellar reactions.

$K_{obs} = K_{micelle} = K_2[Nu] + K_b[OH]$ Eqn 2-2

In most cases, at pH 9.5, the buffer hydrolysis (K_b) is negligible. By keeping the substrate concentration constant, the apparent second order rate constant for the overall process was obtained from the slope of K_{obs} versus [nucleophile]. In general, all reactions followed a linear first order process dependent of nucleophile concentration (at least up to the CMC of the ECNR). Other treatments of kinetic data are specified under the specific examples.

Triester hydrolysis by ECNR

The hydrolytic efficiency of ECNR against an organophosphate triester was studied prior to their action with diester anions. Paraoxon has been previously used[15,27] as a good model substrate. The pH of the medium was set at 9.4, sufficiently alkaline is to have significant amounts of the active nucleophilic (anionic) form of ECNR, yet mild enough that buffer hydrolysis of the triester be negligible. Paraoxon (23) is a structural analogue of alkyl (or aryl) 4-nitrophenyl phosphate diesters, therefore, the kinetic data of this reaction would be significant for comparison studies. A Paraoxon analogue, octyl methyl 4-nitrophenyl phosphate (24), more lipophilic than Paraoxon, was studied to investigate the influence of the hydrophobic octyl chain on the hydrolytic rate. Other triesters such as testosterone methyl, chloromethyl, 4-nitrophenyl phosphate and testosterone methyl 4-nitrophenyl phosphate were tested against some ECNR in order to evaluate reactivity of these reagents with sterically hindered phosphorus triesters.

Reaction of hydroxamic acid with phosphate triesters

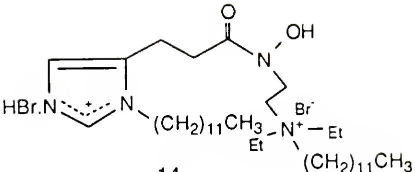
The reaction of Paraoxon (5.5×10^{-5} M) with hydroxamic acid 14 (0.38 to 2.75×10^{-3} M) was carried out at pH 9.3. The kinetic data are summarized in Table 2-3. The pseudo first order rate constant K_{obs}, was obtained from the half life determined from the semilogarithmic plot of A_t-A_∞, where A_t and A_∞ are the absorbances of p-nitrophenolate at time t and infinite time respectively. At 2.75×10^{-3} M concentration of 14 the reaction was over within 17 minutes. The plot (Figure 2-22) of observed first order rate constants obtained for the reaction at different concentrations of 14 shows a linearity between 0.30 and 1.50×10^{-3} M and a deviation from the linearity around 1.6×10^{-3} M, which is an indication of a micellar acceleration of the reaction. The concentration of 14 at the point of deviation was in close agreement with the CMC measured (2.10×10^{-3} M) using the fluorescence emission method. The acceleration factor of 152 times more than buffer hydrolysis was realized at 2.2×10^{-3} M of 14, at pH 9.4. The second order rate constant for this process obtained from the linear portion of the plot (Figure 2-22) was 14.94 M^{-1} min^{-1}.

The acceleration factor is 300 fold at 25°C which is twice as much as the acceleration at 35°C. This relates to the temperature effect[29,90(c)] that may reorganize the micellar shape to include more bound reactants at low temperature.

Table 2-3: Kinetic data for the hydrolysis of Paraoxon[a] at various concentration of 14 in aqueous buffer pH 9.3 (0.03M).

Conc, M $x10^4$	$t_{1/2}$, min	K_{obs}, min^{-1} $x10^2$ [b]	accn
at 35 °C			
3.75	130.40	0.531	22[c]
7.35	67.62	1.02	42[c]
8.02	62.9	1.11	45
12.4	37.64	1.84	76
13.6	30.65	2.26	93
15.4	26.5	2.60	107
22.9	18.7	3.71	152
28.1	17.7	3.91	168
buffer	2840.4	0.0245	----
BzHA (67) (14.5)	1424.0	0.0487	2
at 25 °C			
1.43	73.9	0.937	211[d]
2.1	52.3	1.33	300[d]
buffer	15623	0.0044	----

a: Paraoxon ($5.5x10^{-5}M$)
b: std deviation was less than 5% of the rate constant listed.
c: buffer (0.01M)
d: buffer (0.02M)



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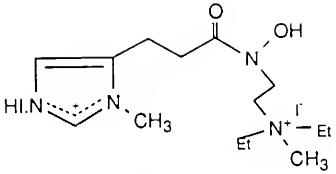

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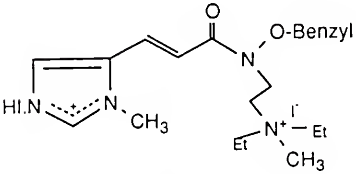

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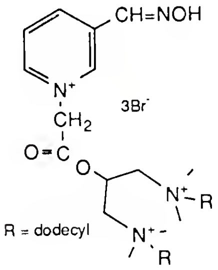
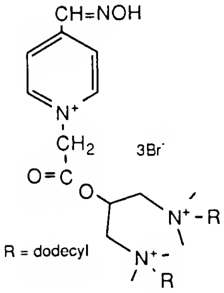
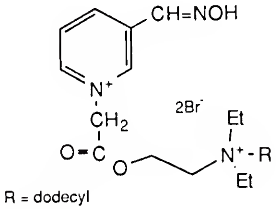


16(b)



19(b)



18(b)

Figure 2-21: The structure of ECNR compounds.

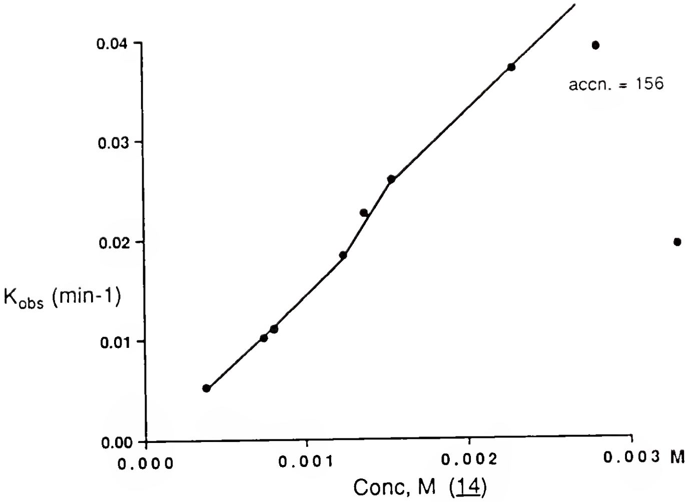


Figure 2-22: Hydrolysis of Paraoxon by hydroxamic acid 14 in pH 9.3 at 35°C.

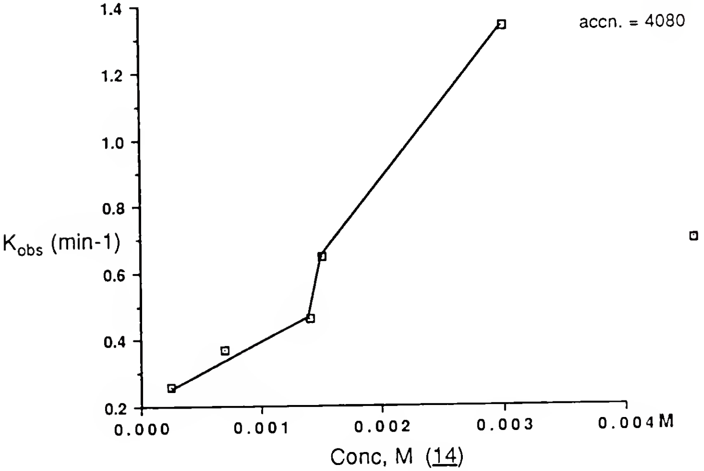


Figure 2-23: Hydrolysis of octyl methyl 4-nitrophenyl phosphate by hydroxamic acid 14 at 35°C.

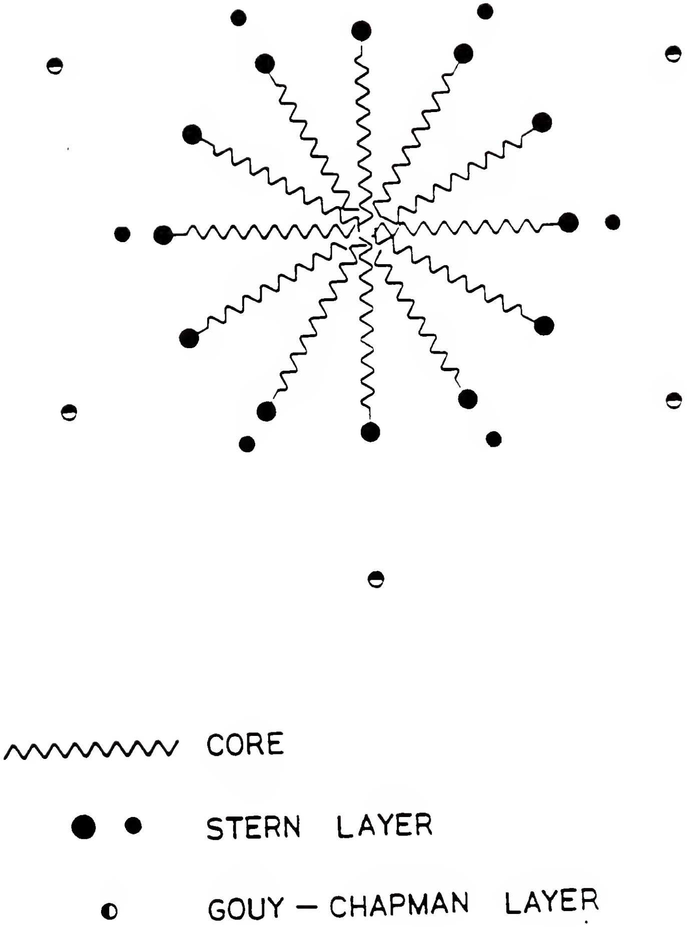


Figure 2-24: Hartley model of micelle.

The reaction of 14 with 24 (octyl methyl 4-nitrophenyl phosphate) is extremely fast and was completed within a few minutes with a magnitude of rate enhancement over 2,000 fold at a reactant concentration of 1.6×10^{-3} M. The apparent second order rate constant for this process calculated from the linear portion of the plot (Figure 2-23) of observed first order rate constant against concentration of 14 was 315.0 M^{-1} min^{-1}. To date, no cationic functional surfactants found in the literature have shown comparable acceleration of the hydrolysis of organophosphates.

Table 2-4: Kinetic data for the hydrolysis of octyl methyl 4-nitrophenyl phosphate[a] by 14 in aqueous buffer pH 9.3 (0.03M) at 35°C.

conc, $\times 10^{4}$M	$t_{1/2}$[b], min	K_{obs}[b], min^{-1}	accn
2.39	2.70	0.257	745
6.88	1.87	0.370	1082
14.0	4.65	0.465	1370
15.0	1.07	0.648	1910
30.0	<0.5[c]	1.386	
49.0 (15)	1113	6.22×10^{-4}	2.55
buffer	2042	3.99×10^{-4}	—

a: octyl methyl PNPP ($5.5-6.5 \times 10^{-5}$M)
b: r = 0.997±0.002
c: extrapolated

The micellar reactions of phosphate esters and carboxylic esters and cationic functional reagents are believed to occur at the Stern region of the generally accepted classical Hartley model[29,91] of the micelle (Figure 2-24). The predominant source for rate enhancement is concentration of reactants into a small volume element. Beside the nature of the functional surfactant the micellar reaction depends, generally, on factors affecting micellar volume and degree of incorporation of reactive entities.[29] It could be suggested that Paraoxon is sufficiently incorporated in the micelle surface to allow appropriate interaction between reactive groups of the phosphate and hydroxamate anion of the micelle in order to give such rate enhancement. The addition of ethanol decreases the micellar volume by increasing the CMC, and therefore decreases the reactivity.[90(c)] The reaction of 14 with Paraoxon can be compared with that of some N-alkyl ß-amino hydroxamic acids in the presence of CTAB[19] from which it can be stated that 14 behaves efficiently as a nucleophile and a cationic surfactant.

The huge rate enhancement of the hydrolysis of octyl methyl PNPP (24) is the result of the hydrophobic octyl moiety. The octyl moiety allows the phosphate ester able to penetrate sufficiently beyond the micellar surface by dissolving in the hydrophobic core of the micelle and thereby allowing excellent incorporation of the substrate. It has been shown[90(c)], in general, that increasing chain length of the substrate enhances the reactant concentration in the micellar Stern layer, and therefore increases the hydrolytic rate. The buffer hydrolysis of 24 at pH 9.5 was somewhat slower than that of Paraoxon under the same condition, which can be reasoned by steric hinderance of octyl group on attack by OH^- relative to non hindered Paraoxon.

Reactivity of N-methyl analogue of 14

Compound 15, the methyl analogue of 14, showed low reactivity (Table 2-5) against Paraoxon and octyl methyl PNPP under conditions used for 14. This result was expected due to the lack of hydrophobic character of 15 needed to form micelles in the aqueous medium. In addition, 15 may exist completely or partially in a zwitterionic form (betaine) in the aqueous medium, as it is evident from the ^{1}H NMR spectrum, and consequently, play a role in reducing the nucleophilicity of the hydroxamate anion.[90b] Comparison of the second order rate constant of 15 (0.087 M^{-1} min^{-1}) with the first order rate constant of 14 shows that the rate enhancement in the functional micelle is caused by the increase in concentration of reactive groups at the micellar surface.

Table 2-5: Hydrolysis of Paraoxon[a] by 15 (non-micellar analogue of 14) in pH 9.5 (0.03M) at 35°C.

Conc, M	$t_{1/2}$, min	K_{obs}, min^{-1}	Accn.
1.23×10^{-3}	2065.13	3.36×10^{-4}	1.4
4.90×10^{-3}	1112.58	6.22×14^{-4}	2.6
6.12×10^{-3}	889.86	7.80×10^{-4}	3.2
buffer	2840.43	2.44×10^{-4}	—

a: 5.5×10^{-5}M

Reactivity of oxime ECNR with triester

The pseudounimolecular kinetic constants of the oxime ECNR are summarized in Table 2-6. All the oxime compounds showed reactivity with Paraoxon, but much slower than the hydroxamic acid 14 at pH 9.4, 35°C. Among all of these compounds, the bis-dodecyl-4-oxime (19(b)) appears to have high reactivity against Paraoxon; the reactivity of 19(b) at 2.76×10^{-3} M can be compared with 14 at 3.75×10^{-4} M. Despite the low reactivity, oxime ECNRs (16(b), 17(b), 18(b) and 19(b)) showed acceleration of hydrolysis of Paraoxon. Similar to 14, the bis-dodecyl-3-oxime (16(b)) showed increased reactivity with octyl methyl PNPP (24). For example, the half life of Paraoxon at 1.47×10^{-3} M 16(b) was 400 minutes, whereas under the same conditions octyl methyl PNPP was hydrolyzed with a half life of 44 minutes.

The relative reactivity (Figure 2-24) of oxime ECNRs against Paraoxon is in the order of Bis-dod-4-oxime (19(b) > Bis-dod-3-oxime (16(b)) > Mono-dod-3-oxime (18(a)) > Bis but-3-oxime (17(b) > 1-nonyloxycarbonylmethyl pyridinium-3-oxime (21) > 1-dodecyloxycarbonyl methyl pyridinium-3-oxime (22).

It is known that 3-oximes exhibit more nucleophilic character than 4-oximes in basic solution. However, the reactivity of 3-oxime 16(b) (pK_a 9.38) was about half that of 4-oxime 19(a) (pK_a 7.69) with Paraoxon at pH 9.4. This can be reasoned by accounting for the free oximate anion[15] concentration available in both cases at pH 9.4.

Table 2-6: Kinetic data of the reaction of oxime ECNRs with Paraoxon and octyl methyl PNPP at 35°C in pH 9.4 (0.03M).

Compd.	Conc, M	$t_{1/2}$, min[b]	K_{obs}, min^{-1}	accn.
Paraoxon (23)[a]				
16(b)	6.21×10^{-4}	633.1	1.0×10^{-3}	4.5
16(b)	1.24×10^{-3}	422.1	1.65×10^{-3}	6.7
16(b)	1.24×10^{-3} [c]	179.6	3.86×10^{-3}	14.0
16(b)	1.86×10^{-3}	254.0[d]	3.11×10^{-3}	12.8
16(b)	3.69×10^{-3}	150.0	4.63×10^{-3}	18.9
17(b)	1.22×10^{-3}	673.0	1.03×10^{-3}	4.28
18(b)	1.24×10^{-3}	539.3	1.29×10^{-3}	5.3
19(b)	1.29×10^{-3}	279.0	2.48×10^{-3}	10.2
19(b)	2.76×10^{-3}	134.8	2.97×10^{-3}	12.2
21	1.33×10^{-3} [c]	325.0	2.13×10^{-3}	8.74
22	1.37×10^{-3} [c]	433.4	1.60×10^{-3}	6.63
buffer	————	2840.4	2.45×10^{-4}	——
Octyl methyl PNPP (24)[e]				
16(b)	9.55×10^{-4}	64.3	1.07×10^{-2}	32
16(b)	2.78×10^{-3}	37.5	1.85×10^{-2}	54.6
17(b)	1.97×10^{-3}	278.7	2.49×10^{-3}	7.4
buffer	————	2041.0	3.39×10^{-4}	——

a: 5.5×10^{-5}M,

b: r = 0.997±0.001, Std deviation 2-5 %.

c: CTAB (3.8×10^{-3}M)

d: buffer(0.05M)

e: 5.0-7.0×10^{-5}M

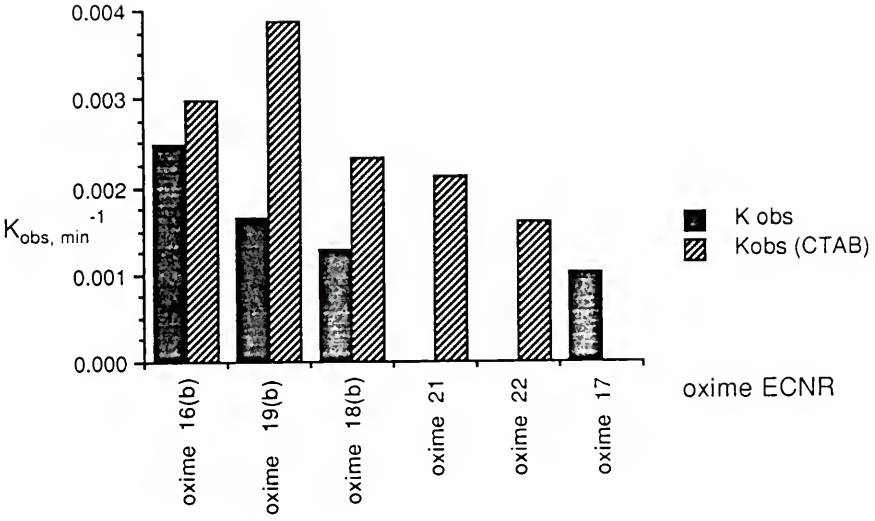
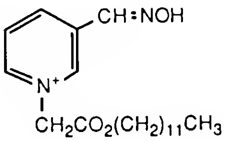


Figure 2-25: Relative reactivity of oxime ECNRs against Paraoxon.

Compound 16(b) dephosphorylated Paraoxon two times faster than did with butyl analogue 19(b), which is due to the fact that short chain butyl group is less effective than the hydrophobic dodecyl group in creating a micellar environment in the aqueous medium.[92(b)] The reactivity difference between bis-dodecyl-3-oxime (16(b)) and mono-dodecyl-3-oxime (18(a)) would correspond to the increased micellar property caused by the double dodecyl group with respect to a single dodecyl chain. It has also been speculated that the increase in cationic moieties or concentration would reduce the pK_a at the pseudomicellar level (medium effect).[93] This may be reasoned for the increased reactivity of oximes carrying bis-(1,3-dodecyl dimethylammonium)-2-propyl moiety relative to those carrying a single diethyldodedcylethylammonium moiety.

It order to investigate the effect of ester (carboxymethyl) linkage as opposed to alkyl linkage of the pyridinium oximes in their reactivity against Paraoxon. Compounds 21 and 22 were prepared as the ester analogues of the 1-dodecyl pyridinium 3-oxime (5). The reaction of 21 and 22 with Paraoxon at 1.22×10^{-3}M concentration and in the presence of CTAB showed a half life of 325 and 433 minutes respectively whereas compound 5[15] under the same condition reduced the half life of Paraoxon to 15-20 minutes. This suggests that the attachement of an a moiety through acyl(carboxymethyl) linkage significantly reduces the overall reactivity of pyridiniumaldoxime against phosphte triesters compared to that by a direct alkyl linkage.



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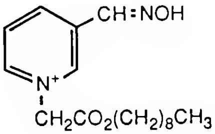

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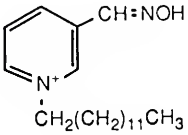

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Effect of added CTAB

In general, CTAB provides a better micellization of reactants or surfactant entities by increasing the micellar volume by breaking monomeric aggregates (turbidity) and solubilizing them in its own micelle or becomes part of the new micelle.[29,90,95] This is generally a hydrophobic effect associated with the transfer (incorporation) of the solubilizate from the water to the micelle and a change in the surface area of the micelle. Therefore, addition of CTAB usually have an additive effect in the reactivity of the cationic micelle.

The results (Table 2-6 and 2-7) indicate that the hydroxamic acid (14) is less influenced by CTAB than are oximes (16(b) to 19(b) on the acceleration of hydrolysis of Paraoxon. For example, Bis dod-3-oxime (16(b)) at 1.24×10^{-3} M concentration showed ≈2 fold increase of rate enhancement, from 1.74×10^{-3} min^{-1} to $3.11 \times 10^{-3} min^{-1}$, on addition of CTAB ($3.9 \times 10^{-3}$ M) whereas hydroxamic acid 14 showed little or no increase of rate enhancement under the same condition.

Table 2-7: Effect of CTAB on the rate of reaction of 14 with Paraoxon[a] at pH 9.3[b].

Conc, M $\times 10^4$	CTAB, M $\times 10^3$	$t_{1/2}$, min	K_{obs}, min^{-1} $\times 10^2$	accn
8.02	——	62.9	1.10	42
7.74	2.0	41.2	1.66	68
7.68	3.98	36.54	1.90	77
1.49	3.85	27.25	2.54	100
1.36	——	30.65	2.26	93
2.59	3.85	16.59	4.18	171
2.68	——	19.96	3.48	142

a: paraoxon (5.5×10^{-5}M)
b: buffer (0.03M), 35 °C

These observations can be explained by the fact that the hydroxamic acid 14 is almost effective as CTAB in providing solubilization of substrate. The oximes 16(b)-18(a) are less nucleophilic micellar reagents than 14 and therefore, the addition of CTAB helped to provide improved incorporation (solubilization) of the substrate in the micellar layer. Incidentally, compounds 21 and 22 are less water soluble than their ammonium ion counterparts, therefore, the use of CTAB was unavoidable to bring about micellization.

The pH-rate profile of reaction 14 with Paraoxon

An investigation was conducted to determine the effect of solution pH on the reactivity of 14 against Paraoxon. The data in Table 2-8 show an apparent increase in rate constants as the pH of the solution was made alkaline. The plot of pH against the rate constants at a constant concentration (2.3×10^{-3}M) of 14 indicates a pH dependent region between pH 6-7 and a pH independent region over pH 10. It is clear from the pK_a value (7.58) of 14 that above pH 10 the compound would would exist as in hydroxamate anion (nucleophile) form.

Unlike a typical aqueous second order nucleophilic reaction, the correlation plot of observed second order rate constants (corrected for buffer hydrolysis) against α_{HA} (degree of dissociation measured by the photometric pK_a measurements) did not show a line through the origin.

$$C_D = C_T - CMC \qquad \text{Eqn 2-3}$$

$$\text{Monomor} \rightleftharpoons \text{Micelle}$$

The relationship of the analytical concentration (C_T) with the micellar bound concentration (C_D) of the reagent can be given by equation 2-3. Since the C_D depends on the dynamic equilibrium of the monomer - micelle exchange governed by many parameters such as temperature, pH, chemical nature of the buffer etc.[96(a)], the change of pH would also change the C_D for a given concentration of C_T. Fendler[96(c)] stated, for a second order reaction, establishing the effective nucleophilic concentration or the proportion of the micellar bound nucleophile in the micelle at different pH is a particularly thorny problem. Thus it can be suggested that for a given nucleophilic [hydroxamate anion] concentration the reactivity can be different at different pH because of the different CMC that may exist at each pH. Consequently, if one varies the pH, or concentration or chemical nature of the buffer, the value of the bound counter ion/free counter ion of detergent, and pK_a at given C_D will also change.[96]

Table 2-8: The reactivity of hydroxamic acid 14 with Paraoxon[a] at various pH buffers (0.03M) at 35°C.

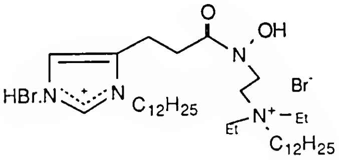
conc, M	pH	K_{obs} [b], min^{-1}	α^{c}_{HA}
2.46×10^{-3}	11.2	5.18×10^{-2}	1.0
2.46×10^{-3}	10.4	4.7×10^{-2}	0.96
2.29×10^{-3}	9.3	2.10×10^{-2}	0.91
2.10×10^{-3}	8.6	1.40×10^{-2}	0.85
2.23×10^{-3}	8.03	1.15×10^{-2}	0.70
2.46×10^{-3}	6.70	2.04×10^{-3}	0.33

a: Paraoxon (5.5×10^{-5}M)
b: corrected for buffer hydrolysis
c: degree of ionization (spectrophotometry)

Mechanism of reaction of 14 with paraoxon

The reaction between 14 and Paraoxon was examined for stoichiometry. The plot (Figure 2-26) of log [Paraoxon] (or log (A_t-A_∞) against time for the reaction of hydroxamic acid 14 ($2.60\times10^{-4}M$) with Paraoxon ($1.90\times10^{-4}M$) at a ratio of 1≈1 showed a rapid initial rate, and following the consumption of reagents the reaction rate slowed down sufficiently. A plot of 1/[paraoxon] against time showed a linear correlation (r=0.998). The second order rate constant (17.63 M^{-1} min^{-1}) obtained for this reaction was comparable with that obtained (14.94 M^{-1} min^{-1}) from the plot of K_{obs} versus time (Figure 2-11). These observations imply that the reaction of 14 with Paraoxon is a bimolecular process.

To investigate whether the basic pK_a of the imidazole group of compound 14 is affecting the reaction rate at pH 9.5, benzyl hydroxamate 67 ($1.87\times10^{-3}M$) was reacted with Paraoxon (5.5×10^{-5}). The reaction showed a rate that is about 20 times slower than the rate of hydrolysis of Paraoxon by 14. This suggests that the imidazole moiety of 14 plays little or no role in the hydrolytic reaction of Paraoxon at pH 9.5.



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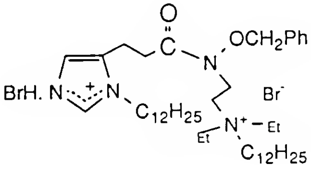

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The pH-rate profile, the reaction of stoichiometric concentration of reagents and the reaction 67 with Paraoxon clearly demonstrae that the hydroxamic acid 14 is not a true catalyst, but rather a nucleophilic reagent. The intermediate hydroxamate-phosphate is more stable,[97] no significant amount of the 14 are regenerated. Additional evidence was obtained from non micellar reaction of 15 with Paraoxon, where the linear regression of observed rate constant against concentration at a fixed condition (pH 9.3, 35°C) gave a satisfactory linear relationship (r = 0.989) through the origin.

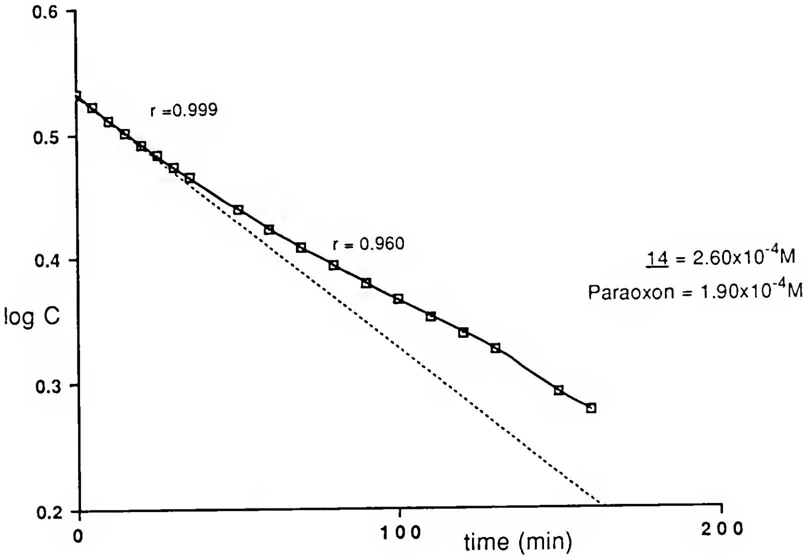


Figure 2-26: Hydrolytic rate of Paraoxon by hydroxamic acid 14 under non first-order reaction conditions.

Phosphoester monoanion hydrolysis by ECNR

Finally, principal kinetic studies of these cationic nucleophilic reagents were made on series of phosphate and phosphonate monoanions to evaluate the major hypothesis of the main objective. A detailed examination of the hydrolysis of phosphoester monoanion as the model aged acetylcholinesterase enzyme (AChE) by ECNR will provide an indication of the potential usefulness of this approach in designing similar multicharged nucelophiles for in vivo use. So far the observed hydrolysis of diester phosphate and phosphonate monoanion reported[10,11,14,35,99] in the presence of various nucleophiles under various conditions are much slower than the OH^- catalysed hydrolysis of the corresponding triesters.

A series of phosphate diesters anions (26-33) carrying 4-nitrophenyl group and various sizes of alicyclic moieties were examined for the hydrolysis in the presence or absence of the reagent. Kinetic data were based on the production of 4-nitrophenolate or 2,4-dinitrophenolate anions.

In view of the difficulty in determining the phenoxide production rate for slow reactions, kinetic constants were either obtained by "initial method"[98] or obtained from the semilograthmic plot of $A_\infty - A_t$ versus time, where A_∞ is the estimated absorbance of phenoxide >95% completion of reaction and A_t is the absorbance at time t. Estimation of A_∞ for the semilograthimic method was made from the calibration curve. In the initial method the absorptive coeffecient (ϵl) of 17925 $M^{-1}\ dm^3$ was used for 4-nitrophenol in buffer pH = 9.4, which was obtained from the calibration curve of absorption against concentration. The initial rate method was found to be more convenient than the semilograthmic method for measuring kinetic constants of very slow reactions whose half life is more than a couple of days. In general, both methods are used and the kinetic constants obtained were compared.

Hydrolysis of bis-4-nitrophenyl phosphate (32) and bis-2,4-dinitrophenyl phosphate (33)

Bis-4-nitrophenyl phosphate (32) hydrolysis. Compound 32 was found to have a half life of 670 days in aqueous solution of pH 9.4, at 35°C. Trogler et al.[98] reported a half life of 332 days at 75°C in pH 6.6 and Kirby and Younas[10] obtained half life of 130 days at 100°C at pH 7.6.

Compound 14 was reacted with bis PNPP sodium salt (32), under pseudo first-order conditions. The kinetic constants are given in Table 2-9. The plot (Figure 2-27) of pseudomononuclear rate constants at various concentrations of 14 (3.0 to 52.8x10^{-4} M) showed give a linear correlation. The half life of 32 was brought to 740 minutes from 673 days at a concentration of 5.28x10^{-3} M of 14, which corresponds to a rate acceleration of 1,300 fold. Evidently, the reactivity of the hydroxamate anion of 14 with 32 is four times greater than the OH^- hydrolysis of Paraoxon at pH 9.4.

It is noteworthy that the reaction generates mono 4-nitrophenyl phosphate, which is then hydrolyzed slowly to its orthophosphate. In general, under these hydrolytic conditions, the plot of $A_\infty - A_t$ against time would be a biphasic plot. However, no significant effect of mono 4-nitrophenyl phosphate hydrolysis was seen to interfere with the kinetic data acquired over three hours.

The second-order rate constant obtained for the reaction between 14 and 32 time is 0.142 M^{-1} min^{-1} (r= 0.99). Trogler et al.[98] have recently reported that a cupric complex of 2,2-bipyridine hydrolyzed 32 in aqueous solution at pH 8.00 about 2,000 fold times faster than the buffer alone, with an apparent second order constant of 1.2 M^{-1} min^{-1}, at 75°C. Menger et al.[35] have obtained hydrolysis ($t_{1/2}$=215 min) of 32 with a cupric ion complex of a diamine (22 times excess over substrate) at pH 8.5, 25°C. In the case of 14 (5.28×10^{-3} M) at 10 fold excess over the concentration of 32 showed a rate enhancement of 1,325 fold at 35°C in pH 9.5.

Table 2-9: Kinetic data for reaction of bis 4-nitrophenyl phosphate[a] with hydroxamic acid 14 in aqueous buffer pH 9.3 (0.03M) at 35°C.

conc, $\times10^{-3}$ M	$t_{1/2}$, min[b]	K_{obs}, min^{-1} $\times10^{4}$	accn
1.30	2104	3.30	492
1.30[c]	13353	5.19×10^{-2}	---
1.43	1920	3.61	538
2.60	1245	5.56	800
2.60[c]	2104	3.29	---
3.76	1093	6.33	946
4.89	866	8.00	1119
5.28	732	9.47	1324
buffer+CTAB	---[d]	----	----
buffer	673 days	----	----
16	---[e]	----	----

a: Bis PNPP (5.5×10^{-5})
b: std deviation <5%, r = 0.992±0.002
c: CTAB (3.38×10^{-3}M)
d: no monitorable reaction (inhibition)
e: reaction is as slow as buffer

Unexpectedly, the oxime ECNR 16(b) and 18(b) failed to show any appreciable rate enhancement for the hydrolysis of 32 in the presence or in the absence of CTAB. The rate of hydrolysis of 32 by 16(b) is found to be as slow as the buffer alone even at concentration as high as 3.5×10^{-3} M. The oximate anion had an appreciable absorption at 400 nm, consequently, no quantitative measurements for the slow release of phenoxide ion (reaction rate) can be made.

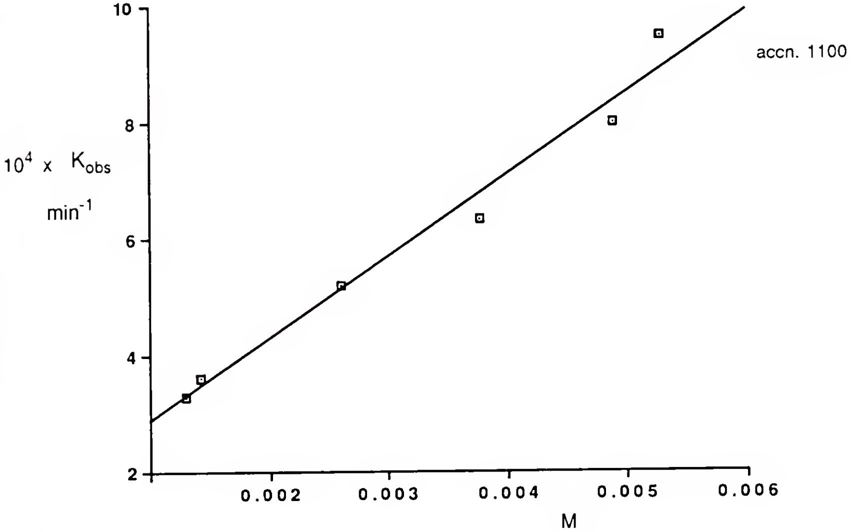
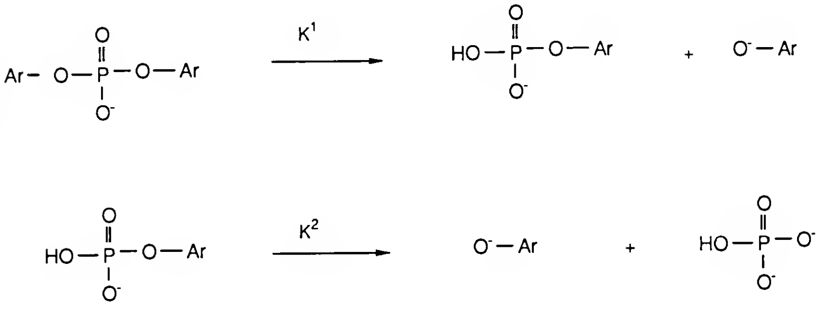


Figure 2-27: First-order rate constants for the hydrolysis of bis 4-nitrophenyl phosphate (32) at various amount of 14 in pH 9.5 at 35°C.

Bis(2,4-dinitrophenyl) phosphate (33) hydrolysis. Micellar effects upon the hydrolysis of bis-2,4-dinitrophenyl phosphate (33) have been studied extensively by Bunton, et al.[74,102] It has been shown that the hydrolysis of 33 is a two step process, where k^1 and k^2 are first-order rate constants with respect to the phosphate ester, and at high pH (>9.0) $k^1 >> k^2$, and the corresponding reaction of 2,4-dinitrophenyl phosphate dianion makes less contribution to the overall reaction. In the presence of an excess of effective nucleophile the initial reaction, the nucleophilic attack of the phosphate diester monoanion, would be faster than the second stage. Thus the kinetic data obtained for the initial part of the reaction corresponds to the diester monoanion (33) hydrolysis.



Eqn 2-4

The plot of log A_t against time for the reaction of 14 with 33 at different concentrations of 14 and at different pHs (7.0-9.5), is biphasic, where a rapid phase I reaction followed by a slow phase II reaction representing diester monoanion and monoester dianion hydrolysis respectively. In order to determine the precise end point of phase I a calibration plot, for the absorbance (350 nm) of phenoxide ion at different concentration of 2,4-dinitrophenol in a buffer of interest, was used. Another method involved addition of alkaline phosphatase enzyme to the aged reaction mixture in small quantity, which rapidly hydrolyzed the remaining mono 2,4-dinitrophenyl phosphate, and the UV spectrum of this solution gave the final A_∞ of the complete (phase I & II) reaction.

The kinetic constants for the hydrolysis of 33 in aqueous solution of pH 9.4 ± 0.1 are summarized in Table 2-10. The reaction of 14 with 33 is extremely rapid, and the reaction was completed within one to two minutes at ten fold excess of 14. At a concentration of 14 ($1.23\text{x}10^{-4}$ M) which is about two fold lower than its CMC, the reaction gave 2,540 fold rate enhancement, the half life of 33 was less than 45 seconds. Beyond this concentration of 14 ($1.30\text{x}10^{3}$) the reaction half life was very short (<30 sec), which caused an instrumental limitation to obtain more accurate kinetic data. Extrapolation of the kinetic data from Table 2-10 to a concentration ($2.30\text{x}10^{-3}$ M) of 14 gave a rate enhancement of 4,600 fold. An apparent second-order rate constant obtained from the pseudo first-order kinetic data for the phase I reaction was found to be ≈ 1,304 M^{-1} min^{-1} at 35°C in pH 9.4. This magnitude of rate enhancement below the CMC of a micellar reagent is rare in micellar chemistry. However, this can be explained by the formation of submicellar aggregates[102] below the CMC, where the micellar model often fails to explain the phenomena. It is also noteworthy that reaction of 14 with monoanion 33 is a lot faster than is its reaction with neutral Paraoxon by about 15 to 20 fold.

Table 2-10: Kinetic data for the biphasic reaction of 14 with bis-2,4-dinitrophenyl phosphate[a] (33) in aqueous buffer pH 9.4, 8.0 and 7.0 at 35°C.

comp, M	$t^1_{1/2}$[b]	$t^2_{1/2}$[c]	$K^1_{1/2}$[b]	$K^2_{1/2}$[c]	accn[b]
$x10^4$	min		min^{-1}		
pH 9.4 (0.03M)					
5.73	1.06	150.35	0.653	$4.61x10^{-3}$	1010
12.30	0.46	35.15	1.51	$2.72x10^{-2}$	2541
22.26	<15sec	>2.80			>4600
CTAB[d]	198.1	----	$3.50x10^{-3}$		6
buffer	1169	----	$6.23x10^{-4}$		----
pH 8.0 (0.05M)					
5.73	3.97	----	0.198	---	3181
buffer	11,132	----	$6.23x10^{-5}$		----
pH 7.0 (0.05M)					
5.73	6.8	----	0.102	---	
buffer	----[e]	----			

a: Bis 2,4 DNPP (4-5x10^{-5}M)
b: kinetic constants for phase I reaction
c: kinetic constants for phase II reaction
d: CTAB (3.92x10^{-3}M)
e: no apprarent reaction over 2 days.

Evidently, phase I reaction of 33 with 14 was rapid even at pH 8.0 and 7.0, but less rapid than at pH 9.5, which is understandable if one considers the effective nucleophilic concentration of 14 at the relevant pHs. The acceleration of 14 at pH 7.0 was very remarkable, where the half life of 33 was brought down to about 4 minutes from 7.7 days in the buffer to give 3,200 fold rate enhancement.

The phase II reaction showed a pseudo first-order rate constant 50-75 times slower than that of phase I at pH 9.4. Fendler, et al.[103] showed this ratio to be ≈ 80 (k^1/k^2) for the reaction of 33 with hydroxyl anion in CTAB micelle. The bimolecular rate constants reported[74] for reaction of OH^- with 33 in the presence of CTAB were k_2^1 ~ 0.18 M^{-1} min^{-1} and k_2^2 ~ 0.024 M^{-1} min^{-1} and these values can be compared with reaction of 14 with 33, where $k_2^1 \approx 1304$ m^{-1} min^{-1} and $k_2^2 \approx 22.1$ m^{-1} min^{-1}. The difference in k^1 and k^2 can be explained by the difference in the reactivity between the diester monoanion and monoester dianion with a nucleophile. In monoester dianion hydrolysis the driving force is departure of the 2,4-dinitrophenol (or nitrophenol) group provided by the double negative charge of the phosphate residue.[102] Kirby and Varvogils[103] pointed out that the nucleophilicity of the reagents may not play an important role in the rate-limiting step in the spontaneous (SN_1 type) hydrolysis of 2,4-dinitrophenyl phosphate dianion, but depend strongly on the basicity of the leaving group.

The bis nitrophenyl phosphates such as 32 and 33 are used not as models models of aged acetylcholinesterase enzyme but, to investigate the nucleophilicity of the ECNR with diester anions that have two good leaving groups. It is clear from the above investigation that the effect of negative charge of the anion to inhibit or repel the attacking nucleophile is far greater than is the effect of a good leaving group in the case of bis-4-nitrophenyl phosphate (32). However, in the case of 33 the powerful electron attracting groups of 2,4-dinitrophenyl phosphate can minimize the importance of the repulsion. Additionally, the rate difference between the reaction of 14 with triester 24 (Paraoxon) and 32 may support the observation made by Kirby and Younas[10] that the basicity of the leaving group is actually less important for the diester anion hydrolysis. The reactions of 14 with diesters having two good leaving groups appear to be in the range of expected bimolecular nucleophilic substitution. In addition to submicellar effect the overall reactivity can be explained by the interactions of cations with the anionic substrate.

Hydrolysis of alkyl and aryl phosphorus monoanions

Aged enzyme is well represented by alkyl or aryl 4-nitrophenyl phosphates and phosphonates of various chain lengths and sizes. It was expected that the degree of diversity in the structure and polarity of the diester molecules within the general frame work would provide pertinent kinetic data corresponding to their interactions with the cationic centers of the reagents. The kinetic consequence of a series of diester phosphates, such as dodecyl, octyl, butyl and benzyl 4-nitrophenyl phosphate as well as phenyl 4-nitrophenyl phosphonate, with ECNRs were investigated in aqueous buffer solutions.

Dodecyl 4-nitrophenyl (26) hydrolysis. The pseudo first-order constant for the hydrolysis of 26 by 14 is given in Table 2-11. It was expected that the hydrophobic chain of 26 would help to incorporate the substrate in the micellar Stern layer better than short chain analogue, resulting in some rate enhancement. However, the opposite effect was observed. Although the reaction of 14 with 26 was considerably faster than buffer hydrolysis, only a modest rate enhancement (26-37 fold) was obtained when 14 was used slightly above its CMC. The plot (Figure 2-28) of pseudo first order rate constant against concentration of 14 showed a linear correlation below and above the CMC of 14 and only a slight deviation from linearity around the CMC point was evident for the micellar effect.

The apparent second order rate constant of 0.0391 m^{-1} min^{-1} ($r = 0.996$) was established from the linear portion of this plot, and this value was compared with that obtained, 0.056 M^{-1} min^{-1} from the reaction of 14 (5.714×10^{-4} M) against 26 (1.74×10^{-4} M) performed at 4:1 molar ratio. The oxime ECNRs, 16(b) and 18(a) showed only a few fold rate enhancement of hydrolysis of 26, even at concentrations as high as 3.5×10^{-3} M. The major problem in the kinetic experiments of oxime ECNR is the interference of the absorption spectra of oximate anion at 400 nm, which hampered accurate measurements of phenoxide formation of slow reactions. Compound 19(a) had significant absorption at 400 nm at pH 9.5, which prevented accurate rate constants for its reaction with 26 being obtained.

Table 2-11: Kinetic data for the hydrolysis of dodecyl, octyl, butyl and benzyl 4-nitrophenyl phosphates by 14 in aqueous buffer pH 9.5 at 35°C.

conc, M x10^{4}	CTAB, M x10^{4}	$t_{1/2}$, day^{b}	K_{obs}, min^{-1} x10^{5}	accn
2.88	----	9.65	5.01	11.1
2.88	8.25	253	1.91x10^{-6}	----
5.71	----	7.59	6.34	14.1
5.71	8.25	16.7	2.88	6.4
19.03	9.77	4.5	10.55	24.0
20.0	----	4.08	11.79	26.2
24.7	----	3.17	15.18	33.7
39.2^{c}	----	2.12	22.75	139.4
buffer	----	107		
25.1 (octyl PNPP)		2.3	20.2	
23.8 (benzyl PNPP)		4.11	11.7	
16.4 (butyl PNPP)		0.86	55.8	

a: dodecyl PNPP. lutidine salt (7.0-8.0x10^{-5}M)
b: r = 0.99±0.01
c: dodecyl hydrogen PNPP

The low reactivity of long chain 3-nitro-4-alkoxyphenyl trimethylammonium iodides was accounted for by the increase liphophilicity of the molecule that increased solubility in the micellar Stern layer.[104] Evidently, the electrophilic substrate carrying hydrophobic group would be deeply buried in the Stern layer, thus shielding itself from the nucleophilic attack.[102] This consideration suggests that low reactivity of dodecyl, octyl or even butyl 4-nitrophenyl phosphate in each case can be attributed to deep penetration of the alkyl group of the ester into the micelle, in which the phosphoryl group would be positioned away from the hydroxamate ion, rendering them inactive. Consequently, the phosphoryl group is far out of reach for any electrostatic intractions by the cationic centers to reduce its repulsive charges.

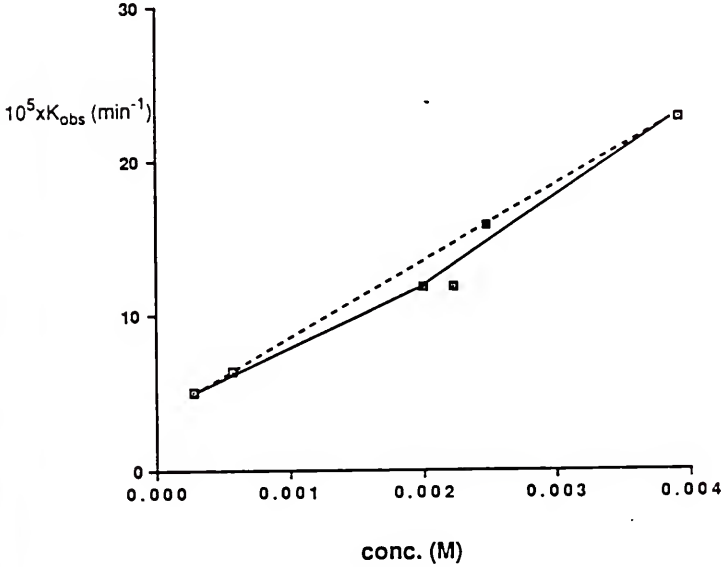


Figure 2-28: Hydrolysis of dodecyl 4-nitrophenyl phosphate (26) by 14 in aqueous buffer pH 9.5 at 35°C.

Romstead et al.[93(a)] and Bunton and Robinson[100(a)] have used similar arguments to explain the rate maxima observed in the reaction of 4-nitrophenyl diphenyl phosphate and hydroxyl anion with increase in concentration of CTAB. Fendler[29] suggested that if a micellar rate enhancement does not exceed 100-200 fold in aqueous solution this can be attributed, in part, to the inability of the micelles to hold the substrate in a rigid form. Romstead[93(b)] stipulated that if reactants and counter ions are hydrophobic the pseudo micellar model fails to explain the kinetic consequences.

Effect of other factors

CTAB on diester monoanion hydrolysis. The reaction of ECNRs with diester anions (26-32) with 32 showed an adverse effect on addition of CTAB. For example, the half life of 32 in the presence of 14 ($2.60\text{x}10^{-3}$ M) was 1332 minutes, and the reaction was inhibited on addition of CTAB ($3.58\text{x}10^{-3}$ M) to raise the half life to almost double (2140 minutes). The inhibition of CTAB was more pronounced at concentrations of ECNR below its CMC, and when CTAB is used above its CMC ($2.4\text{x}10^{-3}$M)[15] in which the reactions were completely halted. No apparent change in reaction rate can be observed with reactions of oxime ECNRs in the presence of CTAB.

$$Br_w \quad + \quad X_m \quad = \quad Br_m \quad + \quad X_w \qquad \text{Eqn 2-5}$$

Few examples,[95,100] such as the inhibitory action of CTAB in the reaction of hydroxide ion with 2,4-dinotrochlorobenzene are found in the literature. The inhibitory action can be explained by an ion-exchange model;[101] where Br_w is the concentration of Br^- in the aqueous phase, and X_m is the concentration of anionic substrate in the pseudomicellar phase, in which Br^- of CTAB would compete with the anionic substrate for a place in the Stern layer and later at a high concentration of CTAB this process would be more vigorous to bring about complete inhibition. Another explanation would be the competitive incorporation of substrate by CTAB and ECNR, from which it appears that CTAB binds to the substrate more firmly than does ECNR, thus resulting in the inhibition of hydrolysis.

Turbidity. It was found that the reaction mixture formed a turbid solution with an increase in buffer strength (above 0.03 M). Turbidity was particularly prominent with the long chain diester phosphate (26 and 27) solutions. The turbid formation at high ionic concentration have previously been observed[94,100(b),102] with micellar solutions, and explained as the formation of sparingly soluble ion pairs between cationic micelle and the basic substrate or probably due to the "salting out" of sparingly soluble aggregates. The turbidity was reduced by the addition of a small amount of CTAB ($\approx 4.0 \times 10^{-5}$M), where CTAB provides dissolution of insoluble premicellar aggregates and incorporate them in the new micelle.

However, addition of CTAB above the substrate concentration resulted in serious inhibition of the reaction (eg., 26 and 32). It seems that the inhibition at very low concentration of CTAB is not due to the competition of substrate anion with Br^- for a place in the ion-exchange micellar model, but to the extensive solubilization of substrate deep in the micelle, preventing it further from any attack by the nucleophile.

Hydroxyl anion hydrolysis of alkyl 4-nitrophenyl phosphates

The buffer hydrolysis of diester anions is an extremely slow process at conditions employed. However, significant differences in the spontaneous hydrolysis were observed with long chain 4-nitrophenyl phosphates with increase in buffer concentration. For example, increase of buffer concentration from 0.03 M to 0.054 M reduced the half life ~107 days to 27 days respectively in pH 9.4. In addition, the results also show a sharp difference in the half life of lutidine salts compared to the hydrogen phosphate form of the diester. This may be explained by comparing the micellar like property of the anionic micelle such as dodecyl sulfate sodium salt (NaS) with alkyl 4-nitrophenyl phosphate such as 26. Thus, the buffer hydrolysis of 26 was enhanced by its own micellar character.

Ethyl 4-nitrophenyl phosphate (29) hydrolysis

Compound 29, an aged enzyme model of the Paraoxon inhibited AChE, reacted with 14 considerably faster than its long chain analogues. The kinetic data for this reaction are given in Table 2-12. At a concentration slightly above its CMC, 14 showed hydrolysis of 29 with a rate enhancement of 550 fold at pH 9.4, the half life of 29 was reduced to 900 minutes from 342 days. The plot (Figure 2-29) of k_1 (pseudo first-order constant) against C_D (concentration of 14) shows overall, a linear first order dependence of the concentration of 14. The second order rate constant obtained from the linear portion of the plot was 0.160 M^{-1} min^{-1} (r = 0.99). Oxime ECNR 16(b) at a concentration of $2.8x10^{-3}$ M showed about 290 fold rate enhancement of hydrolysis of 29.

Table 2-13 compares the kinetic rate constants and rate enhancement of 14, 16(b) and other systems so far reported [14(a),98] on the hydrolysis of 29. Very recently, Trogler, et al.[98] have realized 105 fold rate enhancement of hydrolysis of 29 with a cupric bipyridine complex [cu(bpy)$^{2+}$] at 75°C in pH 6.5, whereas 14 showed 600 fold rate enhancement at 35°C in pH 9.5. Bunton et al.[14] have achieved 86 fold acceleration of hydrolysis of 29 with a functional cationic surfactant, hydroxyethyl-2-diethylalkylammonium bromide, in 0.1M NaOH (pH 12) at 25°C.

Table 2-12: Comparison of ECNR reactivity with other reported systems against ethyl 4-nitrophenyl phosphate (29).

Copd.	pH	temp	accn	K_2, $M^{-1}min^{-1}$	Ref.
14 $(2.45x10^{-3})^a$	9.5	35°C	600	0.160	
16(b) $(2.8x10^{-3})^a$	9.5	35°C	≈250	----	
OH^-(0.1M)+ hydroxy CTAB$(3.00x10^{-2}M)$	>12	25°C	≈85	----	14
OH^-(0.1M) + CTAB$(3x10^{-2}M)^b$	>12	25°C	6	----	14
Cu(bypyr)$^{2+}$ $(1.0x10^{-3}M)^b$	6.5	75°C	105	0.66	97

a: lutidine salt of (29)
b: Na salt of (29)

Table 2-13: Kinetic data for the hydrolysis of ethyl 4-nitrophenyl phosphate[a] (29) by hydroxamic acid 14 in aqueous buffer pH 9.5 at 35°C.

conc. M x10^4	$t_{1/2}$[b], min	K_{obs}, min^{-1} x10^4	accn
5.73	1504	4.61	327
13.4	1169	5.93	421
19.4	930	7.45	530
24.5	907	7.63	542
37.0[c]	1108	6.26	1229[c]
28.1 (16)	1658	4.18	297
buffer	342 days		----
buffer[c]	946 days		----

a: ethyl PNPP lutidine salt (12 -14x10^{-5}M)
b: std deviation is within 5-6%
C: ethyl hydrogen PNPP

Although the reaction of 14 with 29 was significantly faster than that with other long chain diester analogues, it appears from the concentration-rate constant profile (Figure 2-29) to have less micellar property than the corresponding reactions of 14 with dodecyl 4-nitrophenyl phosphate (26) and Paraoxon. This increase in reactivity was consistent with the increase in polarity of (29) relative to the long chain alkyl analogue. This suggests that more polar substrates such as ethyl 4-nitrophenyl phosphate (29) are located predominantly in a relatively aqueous region of the micelle than are the less polar substrates, which should also be the preferred location of hydrophilic $N-O^-$ group.[93] The subdued micellar effect of this reaction can be explained by using an ion-exchange micellar model[101], where the more hydrophilic counter ions are less tightly held in the micelle and they are mainly near to the aqueous phase. If the reactants are located in a water-like region (outer Stern layer) the first-order constant (k_1) decreases or increases only slightly upon incorporation in the micelle[100], which should give results more like an aqueous type reaction.

Interestingly, the reactivity of 14 increased with decrease in the chain length or size of the ester substituent, and followed an order of dodecyl < octyl < benzyl < n-butyl < ethyl. For example, at 2.4×10^{-3} M of 14 the half life of these diesters are 6560, 3430, 5920, 1240 and 900 minutes respectively (Figure 2-30). The possible reasons for this trend will be discussed later.

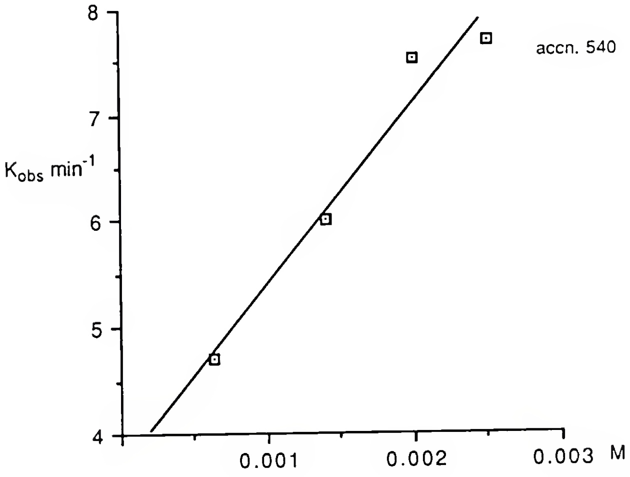


Figure 2-29: Hydrolysis of ethyl 4-nitrophenyl phosphate (29) by 14.

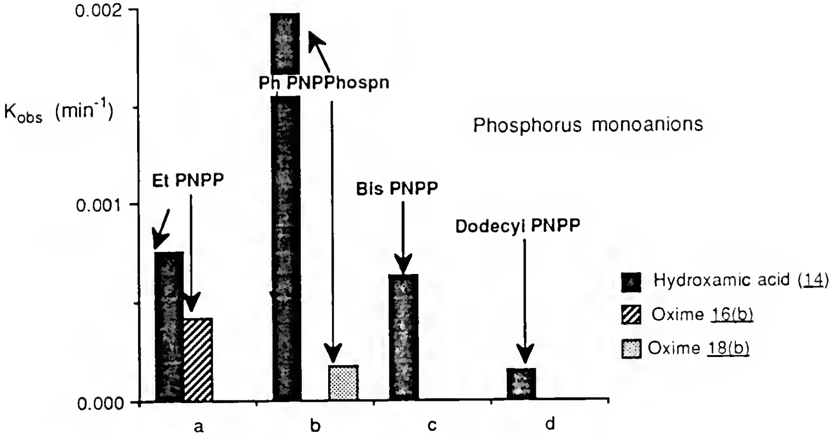


Figure 2-30: Relative reactivity of ECNR against aged enzyme models.

Mechanism of reaction of 14 with diester anions 26 and 29

Figures 2-31 and 2-32 show plots of rate of formation of phenoxide ion (log A_∞ - A_t) against time (t) for the reaction of 14 with 26 and 14 with 29 respectively at a molar ratio of ≈1:2. Evidently, both plots showed rapid initial rate and followed by a slow phase upon consumption of reactants. The plots of $1/A_\infty$-A_t versus time gave a straight line (r=0.997) in each case. The regeneration of 14 from the product intermediate was not apparent under these conditions. These observations demonstrate that the reactions of 14 with 26 or 29 is a bimolecular process. So the kinetic process of 14 with diester phospate monoanions parallel that with Paraoxon.

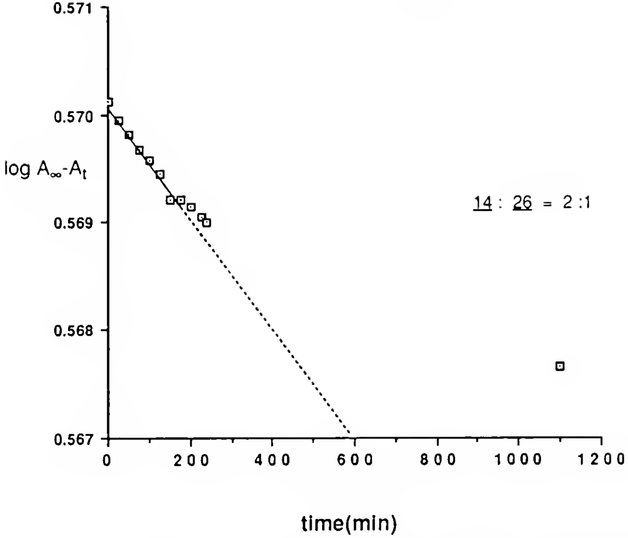


Figure 2-31: Hydrolysis of ethy; 4-nitrophenyl phosphate (29) by 14 under non first-order rate condition.

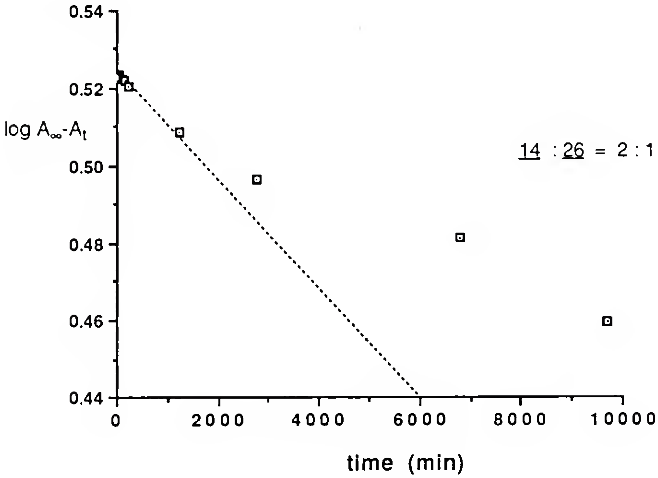


Figure 2-32: Hydrolysis of dodecyl 4-nitrophenyl phosphate (26) by 14 under non first-order rate condition.

Hydrolysis of phosphonate monoanion

Most of the potent nerve agents that have potential application in the field are phosphonate derivatives (e.g., Soman (UD), Sarin (GB), VX, etc). Phosphonates have one of the P-O-C bonds of phosphate esters replaced by P-C bond. An aged model of a phosphonylated enzyme can be represented by a phosphonate monoanion such as 4-nitrophenyl phenylphosphonate (34). For comparison, a diester phosphate monoanion and a phosphonate monoanion are equally resistant to any nucleophilic attack. The rate enhancement for the reaction of 34 with the hydroxyl anion in the presence of CTAB has been realized[14,105] to be only 1.26 fold, in contrast to 6.3 fold for ethyl 4-nitrophenyl phosphate.

4-Nitrophenyl phenylphosphonate (34) hydrolysis by ECNR. Representative pseudo first-order kinetic data are tabulated (Table 2-14) for the hydrolysis of 34 by compounds 67, 14 and 19(b) at pH 9.5. The observation of pseudo first-order kinetics for several concentrations of 14 (Figure 2-33) for the hydrolysis 34 shows a linear relationship with no apparent indication of micellar reation. The pseudo first-order rate constants obtained for this reaction surpassed that observed (or reported) for any phosphate diester monoanions or even phosphonate monoanions. The rate enhancement at 5.0×10^{-3}M concentration of 14 was 1,730 fold, which reduces the half life of 34 from 180 days to 150 minutes. A second-order rate constant obtained form the plot shows a value 0.374 m^{-1} min^{-1} (r =0.997) which is about 2.5 times more than that of ethyl 4-nitrophenyl phosphate (29), 0.160 M^{-1} min^{-1}, and 10 times the value for dodecyl 4-nitrophenyl phosphate (26), 0.0391 M^{-1} mol^{-1}, under the same condition.

The reactivity of the benzyl ether of 14 (67) with 34 is 20 times slower than that observed with 14 at pH 9.5, which indicates clearly that the actual reactive center (nucleophile) is the hydroxamate anion, and not the imidazole moiety. Compound 15, a non micellar analogue of 14, showed appreciable reactivty with 36 relative to buffer hydrolysis. Compound 19(b), 4-oxime ECNR, showed a significant reactivity at a concentration of 2.48×10^{-3} M against 36, and provided a rate enhancement of 64 fold, which can be compared with the reactivity of 14 at a concentration of 10^{-5} M.

Table 2-14: Kinetic data for the hydrolysis of 4-nitrophenyl phenylphosphonate[a] (34) by 14 at 35°C in pH 9.50 (0.03M).

$10^4\times$ M	$t_{1/2}$, min[b]	$10^3\times K_{obs}$, min^{-1}	accn
8.51	596.33(613)	1.269	475
16.71	434.6(477)	1.595	596
24.59	361.9(411)	1.915	716
24.89	351.0(376)	1.971	739
33.34	315.8(347)	2.19	821
50.0[c]	150.0[c]	4.62	1728
20.21 [15]	2.32 days	-	77.6
18.5 [65]	4.05 days	0.11	44.76
24.8 [19(b)]	-	0.102	64.0
CTAB + OH^-	-	-	1.6[105]
buffer	180 days	2.7×10^{-4}	---

a: phosphate anion (8.25×10^{-5}M).
b: std deviation 2-5%, r = 0.995±0.003.
c: extrapolation.
Values in the prenthesis are calculated from "initial rate" method, $\epsilon l = 17{,}925\ M^{-1}dm^3$.

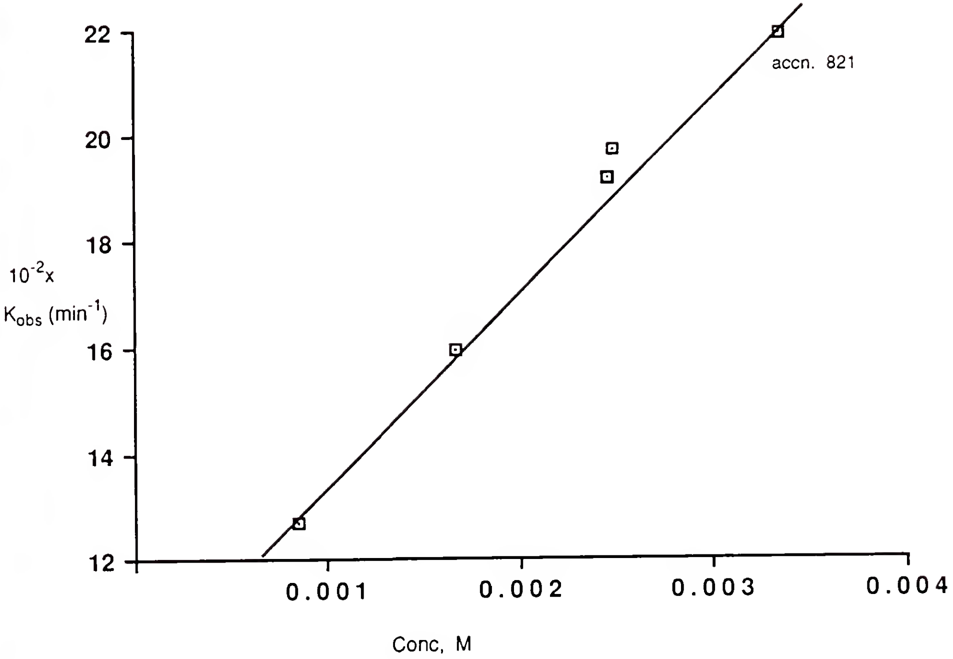


Figure 2-33: Hydrolysis of phenyl 4-nitrophenylphosphonate by hydroxamic acid 14 in pH 9.5 at 35°C.

Substrate Specificity and Mode of Action

The lack of micellar character in the reactions, particularly reaction of hydroxamic acid 14 with phosphorus monoanions, suggest that the reaction takes place predominantly in the aqueous-like phase of the Stern layer that is least affected [97] by the micellar incorporation phenomena. The polar nature of the phosphorus anions 34 and 29 compared to their long chain analogues help to position them in the outer region of the Stern layer (aqueous like region), possibly held close to the proximity of the nucleophilic anion by an effective electrostatic interaction with the cationic residues. This may infer that the electrostatic effect is more pronounced than the solubilization of substrate in the micelle for a effective hydrolysis of phosphorus monoanions.

Oxime ECNRs have proven to be the least effective nucleophiles for the hydrolysis of neutral triester phosphate (e.g., Paraoxon). The low reactivity of oxime ECNR may be due to their highly polar nature and the poor orientation of the reactive groups or lack of cationic binding sites in the proximity of the nucleophile (oximate anion) to invoke attack. Despite these factors the 3-oxime 16(b) and 4-oxime 19(b) showed low to moderate reactivity with ethyl 4-nitrophenyl phosphate (29) and 4-nitrophenyl phenylphosphonate (34). This is more than that observed with reagents such as CTAB/OH^- [67,106], and with cu(bipyr)$^{2+}$ [98].

Their reactivity with phosphate (or phosphonate) monoanions suggests that the electrostatic interactions between the anionic substrate and the cationic moieties of the nucleophilic reagents are more important in diester monoanion hydrolysis than the micellar character of the reagent alone. It appears from the kinetic data that the major driving force to the reaction between ECNRs and diester phosphate (or phosphonate) monoanions is the electrostatic interaction of charges. Micellar effect (solubilization) provided only a modest contribution to the overall rate enhancement. Buist et al.[102] have realized in the base catalysed hydrolysis of bis-2,4-dinitrophenyl phosphate monoanion in CTAB that the reaction requires both the anions come together on a cationic micelle. It may be suggested that favorable electrostatic interactions between the cationic center and the substrate and the dispersal negative charge in the transition state are responsible for, least in part, the increased reactivity.[100,106]

In order to establish a molecular picture for these observations, a molecular model was developed based on a structural analogue of 14 and ethyl 4-nitrophenyl phosphate. Initially, the geometric parameters of the chosen structures were optimized using a semi-empirical (AM-1(Austin Model1)) program run on a VAX computer. A rational fit of the molecules was obtained (Figure 2-34) on feeding the optimized coordinates of the reactant models to a 3-D molecular structure drawing program (Chem3D MM2).

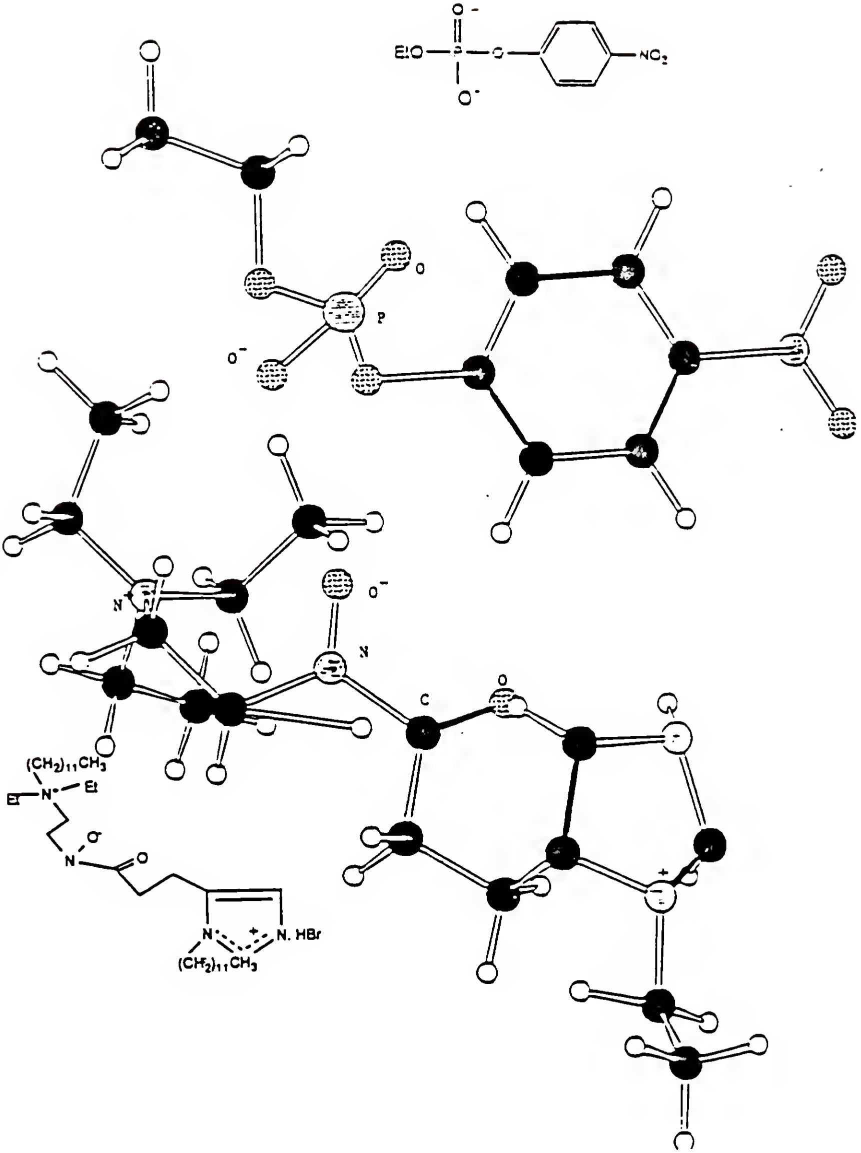


Figure 2-34: A molecular model represntation of the mode of action of 14 with an organophosphorus monoanion.

The molecular model shows that the tetralkylammonium moiety may be involved in the direct electrostatic interaction (neutralization of charges) with the incoming anionic group ($P-O^-$) of the substrate. Thus, the diethylalkylammonium moiety of 14, where R=dodecyl, would exert steric hinderance to the incoming substrate anion and this steric effect is enhanced if the substrate carries bulky groups (e.g., dodecyl). This recognition process would allow relatively small substrates such as ethyl 4-nitrophenyl to bind to the this diethylalkyamonium moiety ("cavity") relatively easier than the bulky substrates like dodecyl 4-nitrophenyl phosphate. However, the O-R groups replaced by a phenyl ring seems to fit into this "cavity" better. The binding of the anionic substrate to the "cavity" would place the phosphoryl group in the proximity of the nucleophilic head ($N-O^-$) that is positioned three atoms away from the binding site and projected towards the aqueous region. The imidazolium moiety of 14 could influence the nucleophilicity of the hydroxamate anion by medium effect and the attached hydrophobic dodecyl group is thought to be taking part in the make up of the micellar environment, which allows to hold the reactive sites in the polar region (water like region) of the micelle. This model explains the enzyme like behavior of 14, which is also rare in the aqueous micellar chemistry.[107] Overall, reaction of ECNR with diester anions supports the theory that effective reduction in electrostatic repulsion and simultaneous nucleophilic attack is an efficient method of dephosphorylation of phosphorus monoanion.

Evaluation of N-Bromo Compounds

Physical-Chemical Studies

Bromine potential

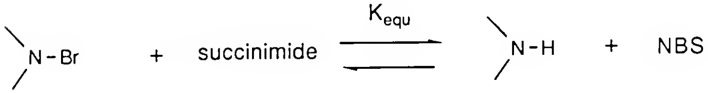
The definition of "bromine potential" (k_{bp}) has been described in Chapter I, and can be quoted as a measure of the ability of an N-bromo compound to release positive bromine in solution (Eqn 2-6). The stability and reactivity of the N-bromo compounds depend largely on the "bromine potential". In general, a reactive N-bromo compound would have a high bromine potential due to its highly polarized N-Br bond, and consequently would be unstable. The stability of an N-halo compound can be increased at the expense of reduced reactivity. The N-bromo compounds having highly labile N-Br bonds are highly unsuitable for use as decontaminants. The key portions of this work are to determine the bromine releasing power ("bromine potential") of the synthesized N-bromo compounds and relating these values with previosuly known compounds such as NBS (37) and 3-bromo-4,4-dimethyl-2-oxazolidinone (43).[45]

$$R_2N\text{-}Br \longrightarrow R_2N^- + Br^+ \qquad \text{Eqn 2-6}$$

$$R_2N\text{-}Br + H_2O \overset{K_{bp}}{\rightleftharpoons} R_2NH + HOBr \qquad \text{Eqn 2-7}$$

$$K_{bp} = \frac{[R_2NH]\ [HOBr]}{[R_2N\text{-}Br]\ [H_2O]}$$

Kaminski and Bodor[45] devised a method that compares the polarity of the N-bromine bond of N-bromo compounds to that in N-bromosuccinimide (NBS) (Eqn 2-8). The "bromine potential" in this case has been defined in terms of the equilibrium constant (k_{equ}) for the reversible reaction between N-bromo compounds and succinimide to form the corresponding free amine and NBS.



Eqn 2-8

$$K_{equ} = \frac{\left[\left(\frac{A_e - A_o}{A_\infty - A_o}\right) \left[\text{N-Br}\right]_o\right]^2}{\left[1 - \left(\frac{A_\infty - A_o}{A_\infty - A_e}\right)\left[\text{N-Br}\right]_o\right]\left[[S]_o - \left(\frac{A_\infty - A_o}{A_\infty - A_e}\right)\left[\text{N-Br}\right]_o\right]}$$

Eqn 2-9

This method involved spectrophotometric determination of the apparent equilibrium constant (k_{equ}) of the reaction of N-bromo compounds with succinimide at a suitable wavelength where neither NBS nor the free amine would have any appreciable absorption. The equilibrium constants (K_{equ}) for the reaction of 3-bromo-4-sulfonyloxymethyl-4-methyl-2-oxazolidinone (47) and 3-bromo-4-succinoyloxymethyl-4-methyl-2-oxazolidinone (49) with succinimide were determined using this method and the values were calculated using the above equation (Eqn 2-9)[45], where A_e has been defined as the equilibrium absorbance at 307 nm, A_0 is the absorbance at 307 nm for the initial concentration of the N-bromo compound and A_∞ is the absorbance at 307 nm assuming a total transformation of N-bromo compound into NBS. Since the initial concentration of N-bromo compounds used in the experiment did not exceed over 10^{-3} M, A_∞ is essentially zero at 307 nm.

The experiments were carried out at neutral to slightly alkaline pHs (7-8) in order to avoid interference from the hypobromite ions (OBr^-), which is produced by the reaction of N-bromo compounds with the aqueous base (OH^-); the OBr^- ions absorb near 340 nm. It is noteworthy that undissociated 47 and 49 show λ_{max} at 269 and 274 nm respectively at pH 8.0.

Table 2-15 shows values of equilibrium constants for the reversible reaction of 47 and 49 with succinimide at various pH. The compounds 47 and 49 showed k_{equ} values 0.33 ± 0.05 and 0.19 ± 0.05 respectively in pH 8.3 at a molar ratio of 1:1 with succinimide. These values can be compared with that previously reported[45] for 3-bromo-4,4-dimethyl-2-oxazolidinone (43), 0.34. If one considers the bromine potential of NBS to be 1.00 the fact that merges from these observations is that the compounds 47 and 49, as well as compound 43 are "soft bromine" (lesser brominating) compounds with respect to NBS and consequently, they exhibit greater stability than NBS.

Table 2-15: Equilibrium constants of the reversible reaction between N-brominating agent and succinimide to form free oxazolidinone and N-bromo succinimide (NBS).

Compd[a]	pH	[N-Bromo]/[succn]	K_{eq} [c.d]
NBMOS (47)	7.5	0.99	0.18
NBMOS (47)	8.3	0.99	0.34
NBMOSuccn (49)	8.4	1.0	0.19
NBO (43)	8.3	1.0	0.33

a: $<3.0\times10^{-3}$M.
b: borate buffer (0.02M).
c: determined at 307nm.
d: values are average of three determinations.

Another observation from these results is that the stability and brominating power 43 is apparently not affected by functionalizing the 4-methyl group of the 2-oxazolidinone system. The succinic derivative 49 seems to have a lower equilibrium constant relative to 47.

The equilibrium state was also found to be sensitive to changes in the pH of the medium. For example, the equilibrium constant for the reaction of 47 with NBS at pH 8.3 was almost double the value of that at pH 7.4. Higuchi et al.[49] have shown in the chlorine exchange reactions of chloramine-T with amines that an increase in pH increased the equilibrium constants of reactions with secondary amines.

The equilibrium constant for dimethylglycouril tetrabromide (54(b)) was not possible to obtain because of insolubility problems. The equilibrium measurement for compound 45 (N-bromo-4-hydroxymethyl-4-methyl-2-oxazolidinone) was not attempted due to its water insolubility (less than 7% w/w) and rapid decomposition (intramolecular) in the solid form. Consequently, compound 45 is unsuitable as an N-brominating agent and thus was excluded from further studies.

Stability of N-bromo compounds

High stability of N-halogen compounds in aqueous solution and solid form are essential if they to be used as commercial decontaminants. However, N-bromo compounds are generally labile in alkaline and acidic media where they are hydrolytically converted back to their parent amine via acid or base catalyzed reaction with water (Eqn 2-7). A requirement for an efficient N-bromo universal decontaminant would be greater stability in these two media relative to conventional type bleach. It is noteworthy that the design of these compounds was based on the fact that compounds having a more stabilized nitrogen lone pair would be less susceptible to acid catalyzed hydrolytic cleavage of the N-Br bond, but more susceptible to base catalyzed clevage,[42] where the latter case is preferred for the stability and function of an efficient decontaminant. It is important to note that the reactive species involved in many of the reactions is the hypobromite ion (OBr^-) and it is formed at basic conditions.

In order to evaluate the overall stability of these brominating agents, the positive bromine concentrations were determined, generally iodometrically[51,108] or in some cases photometrically, in various buffer solutions, and their stability (shelf life) in the solid forms were also determined over a period of time (months). The bromine contents were expressed in percentage relative to their original values in the initial solution or solid form at time zero (Table 2-17). The iodometric titration involved indirect determination of positive bromine by measuring the iodine liberated during the reaction of potassium iodide with the N-bromo compound; the liberated iodine was titrated with standardized sodium thiosulfate solution.[109] The photometric determination of bromine involved monitoring the absorption spectra of the solution of an N-bromo compound over a period of time in the medium of interest. The values were based on the absorbance difference at the $_{max}$ for the N-bromo species.

Examination of the stability data (Table 2-17) for compounds 47, 49 and 59 indicates several interesting observations. The compounds 47 and 49 showed high stability under mild basic and unbuffered neutral conditions relative to acidic buffers (pH < 6). The methylsulfate derivative 47 showed a half life over 112 days in aqueous solution (unbuffered) which is similar to that of 43. This similarity may be attributed to the low bromine potential of these compounds and this trend is apparent from the equilibrium constants (k_{equ}) obtained on reaction with NBS. In addition, compound 47 showed significant stability (9-10 days) in alkaline conditions and even more than that of the water insoluble derivative 43. It appears from these observations that the increased solubility of the methylsulfate N-bromo derivative 47 and N-bromo hemisuccinate 49 did not alter the stability contributed by the structural frame of 43.

However, the succinate N-bromo compound 49 showed less stability in basic solutions, particularly at high pH. For example, compound 49 showed a rapid ($t_{1/2} \approx 40$ min) decomposition at pH 11.43 compared to 47 ($t_{1/2}$=10 days). This may be due to the rapid conversion of 49 to its parent amine and OBr^- ion in alkaline medium. It appears that OBr^- species may be involved in oxidation[111] of the succinate moiety of 49 (or its parent amine) and thus results in loss of positive bromine content.

Compound 47 showed instability in mildly acidic solution similar to that seen[38,44] for the water insoluble analogue 43. The decomposition process of 47 at pH 5.36 and 7.03 followed a pseudo first-order kinetics. A resonable explanation is that the positive bromine species or the hypobromous acid (pk_a 8.69) that was produced under acidic conditions involve spontaneous acid catalyzed decomposition and reaction with the buffer species (CH_3CO_2H or H_3PO_3), and subsequently are consumed in the process. This decomposition process process appears to be faster with phosphate buffer than with acetate buffer.

It is noteworthy that the stability of 47 was significantly altered by the presence of sodium bromide. The sodium bromide contaminated (e.g., bromination of 46 with $NaOH/Br_2$) solutions of 47 showed a reduced stability ($t_{1/2} \approx$ 7-10 days) in the aqueous phase. The accelerating effect of free Br^- ion on the decomposition of N-bromo compounds (e.g., 43) have been realized by Bodor et al.[46] on a solution of CTAB and 43. Thus, the product 47 prepared using t-butyl hypobromite insted of $NaOH/Br_2$ as the brominating agent showed increased aqueous stability ($t_{1/2} \approx$ 20 to 30 days).

Table 2-16: Stability of NBMOS (47), NBMOSuccn (49), tetrabromo dimethylglycouril (54(b)) and NBO (43) (reference) in various buffer solution and in solid form.

Compd	buffer	pH	$t_{1/2}$
47	in water	6.8	≈112 days[a]
47	acetate(0.5M)	5.36	19.2 hrs[b]
47	borate(0.2M)	8.04	>26 days
47	phosphate(0.2M)	7.03	67 mins[b]
47	carbonate(0.2M)	10.50	≈14 days[c]
47	OH^-/carbonate	10,80	≈9 days[c]
49	in water	6.8	[a]
49	borate(0.2M)	8.3	1.75 days
49	carbonate(0.2M)	10.53	2.0 hrs[c,d]
49	OH^-/carbonate	11.43	30-40 mins[c,d]
37(NBS)[108]	in water	---	14.5 days[109]
43	in water	6.8	[a]
43	OH^-/carbonate	11.0	≈30 hrs[109,111]
54(b)	solid[e]	--	≈15.2 months
47	solid[f]	--	≈80 days

a: 5-10% decrease in first 2 days and then no significant change over 7 days.
b: semi logarithmic nature.
c: photometric method.
d: rapid decomposition.
e: solid form stored in the dessicator.
f: solid form stored in the shelf.

The aqueous stability of dimethylglycouril tetrabromide (54(b)) cannot be determined because of its virtual insolubility in water or even in any protic solvents. Despite its aqueous insolubility 54(b) showed an exceptionally high shelf-half life (solid form) of 15.2 months at room temperature, whith respect to half life of 80 days of 47 in the desiccator. Notably, the alkali salts of acids such as 47 and 49 are far more hygroscopic than weak acids such as the urea derivative 54(b).[112]

Stability of ß-hydroxypropyl cyclodextrin formulation of tetrabromo dimethylglycouril (54(b))

The compound 54(b) is potentially an excellent source of bromine, as it would provide four positive bromine species per molecule. In addition, the compound has excellent stability ($t_{1/2} \approx 15$ months) in the solid form. Unfortunately, the insolubility of 54(b) in all organic and protic (except trifluoro acetic acid) solvents seriously hinder its use as a decontaminant. It was of interest to find physical or chemical means to improve the solubility of 54(b) in solvents, especially in aqueous media.

A search for a method to improve aqueous solubility 54 turned to explore the use of hydroxypropyl-ß-cyclodextrin (HPCD) to form an inclusion complex with compound 54(b). Cyclodextrins are cyclic, non reducing oligosacharrides built up from six (α) or seven (β) or eight ( ) glucopyranose units. Cyclodextrins are water soluble, since all of the free hydroxyl groups are on the outer surface of the ring; the internal cavity of the doughnut-shaped portion is slightly apolar. This feature allows inclusion of a guest molecule. Inclusion complexes are entities comprising two or more molecules, in which one of the molecules, the "host" includes, totally or in part, only by physical forces, i.e. without covalent bonding, a "guest" molecule.[113]

Hydroxypropyl ß-cyclodextrin (HPCD) is typical "host molecule" with greater aqueous solubility than other members of the cyclodextrin family. HPCD may complex a great variety of molecules having the size of one or two molecules of benzene and even larger ones carrying a side chain of comparable size, to form crystalline inclusion complexes. This approach has been used successfully to improve aqueous solubility of highly lipophilic therapeutic agents. Brewster et al.[59] have recently reported an example of the use of an HPCD inclusion complex to overcome poor aqueous solubility of a lipophilic carrier drug, estradiol-1,4-dihydrotrigonellinate.

Table 2-17: Stability of tetrabromo dimethylglycouril (54(b)) in hydroxypropyl ß-cyclodextrin (HPCD) formulation.

Formulation	time[e]	pH	% Br retained
50% HPCD[a]	1 hr	--[d]	76.92
	24 hrs		30.76
	48 hrs		11.11
HPCD [b] freez-dry	24 hrs	9.03	8.3
33% HPCD[c] freez-dry	24 hrs	--[d]	35.0

a: 542mg brominating agent in 2mL 50% HPCD.
b: 542mg brominating agent in 2.2mL 33% HPCD.
c: 50.3mg brominating agent in 3mL 33% HPCD.
d: unbuffered deionized water.
e: lapsed time after complexing.

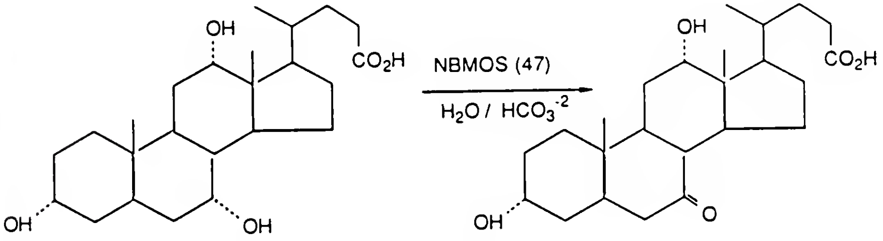
Following a procedure described by Brewster et al.[59(a)], compound 54(b) was complexed with either 50% or 33% HPCD w/v in water to give a completely dissolved solution (0.1 g of 54(b) per 4-5 mL of 50% HPCD). The bromine contents of these complexes with period of time are depicted in Table 2-17. The bromine content sharply decreased ($t_{1/2}$=21 h) on complexation with 50% HPCD complex in water. The freeze dried procedure did not improve the stability of the solid form relative to the solution. In addition, there is no apparent stability difference between complexes made with 50% or 33% HPCD solution. The use of buffered (pH 9.03) HPCD solution resulted in a rapid decomposition of the complex within a few hours.

The aqueous solubility of 54(b) was increased over 100% on complexation with HPCD. However, this complexation also lead to spontaneous decomposition of 54(b). The decomposition may be as the result of oxidation of free hydroxyl groups of HPCD by 54(b) and this process appears rapid with complexes made from buffered HPCD solutions.

Overall, it demonstrate that the inclusion complex of 54(b) and hydroxypropyl-ß-cyclodextrin indeed would increase the aqueous solubility of 54(b), but at the expense of a much reduced stability ($t_{1/2}$ from ≈ 22 months to ≈ 22 hours). Thus, compound 54(b) is not compatible with HPCD in a premade form. Nevertheless, this method could be a good formulation of 54 as a decontaminant with increased active bromine/molecular weight ratio if instant mixing with HPCD could be performed immediately prior to application.

Oxidation reaction of (3-bromo-4-methyl-2-oxazolidinone-4-yl)methyl sulfate(47)

All of the active positive halogen compounds are known to involve oxidation reactions with alcohol moieties to various degrees. In order to investigate the oxidation potency of these water soluble N-bromo oxazolidinone systems relative to NBS (37) and NBO (43), compound 47 was reacted with cholic acid. Selective oxidation of the 7-hydroxy group of cholic acid has been realized with NBS and NBO (43).[40,45] Therefore, it is of interest to dertermine whether NBMOS (47) would selectively oxidize cholic acid under similar conditions.



cholic acid

7-ketocholic acid

Reaction of 47 with cholic acid in the same manner described[40] for NBS gave the corresponding 7-keto cholic acid. The product that was obtained from the reaction was identical to that obtained with NBS and NBO (43) on the basis of ^{1}H NMR, mass spectra, TLC and mp data. However, the yield (30-45%) of the keto product was much less than that observed with NBO (76%) and NBS (67%). The results suggest that 47 is as selective an oxidation agent as NBS and NBS (43), but appears to be less potent with cholic acid.

Decontaminating Effect of Aqueous N-Bromo Compounds

Estimation of OBr^- species production in aqueous solutions of 47 and 49 with various buffers

Table 2-18 depicts the percentage composition of hypobromite ion (OBr^-) to N-bromo species present in equilibrium solutions of compounds 47 and 49 in different buffers. The extent of OBr^- production (equation 1-5) depends on the OH^- concentration in the solution and the nature of the N-bromo compounds. The estimation of OBr^- concentration was based on the absorbance spectra of OBr^- ion at $\lambda_{max} \approx 338$ nm (NaOBr, λ_{max} 350 nm) and the N-bromo compound at $\lambda_{max} \approx 275$ nm. Since the molar absorbances of 3-bromo-4,4-dimethyl-2-oxazolidinone (43) and sodium hypobromite are comparable in basic solution, the percentage composition of these two species was estimated directly from the relative absorbances at their respective λ_{max}.

It is clear from these observations that compounds 47 and 49 in alkaline conditions would be converted to produce OBr^- ions but the extent of conversion is different in each case. Evidently, compound 47 shows large degree of conversion to OBr^- than its succinate analogue 49. Despite the low percentage conversion to OBr^- species, the succinate derivative 49 decomposed rapidly ($t_{1/2} \approx 2$ hrs) than 47 ($t_{1/2} \approx 10$ days) at high alkaline (pH >11) solution. Although 49 showed softer brominating properties than 47 and 43, it appears to be least stable under high concentration of OBr^- ions. The reaction of OBr^- with the succinate side chain could possibly account for this instability. It is was found that the stability (10-12 days) of 47 is higher than the stability of equal concentrations of sodium hypobromite (NaOBr) and 43 under alkaline conditions.

These results suggest that compounds 47 and 49 reacts rapidly with the hydroxide ion in alkaline conditions to yield a mixture with OBr^- ions, and at high pH this equilibrium lies far to the right producing more OBr^- ions, and the stability and percentage conversion to OBr^- of 47 is far greater than 49 in alkaline conditions.

Table 2-18: An estimation of release of active bromine species (OBr^-) in solution of 3-bromo oxazolidinone systems as a function of the solution pH.

pH[a]	47 %NBMOS[b]	%OBr^- [d]	49 %NBMOSuccn[c]	%OBr^- [d]
7.0	---	---	97	3
7.3	86	14	96	4
8.52	59	41	92	8
9.4	---	---	90	10
9.97	57	43	---	---
10.57	46	54	66	34
10.76	37	63	55	45
11.20	28	72	44	56[d]

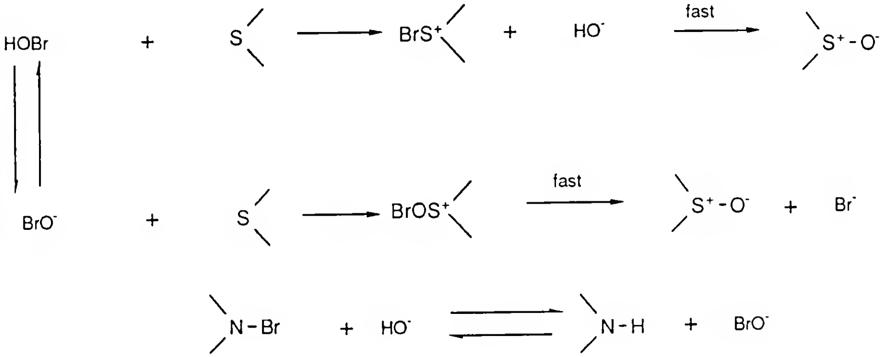
a: buffer concentration, 0.2M, 30 °C.
b: λ_{max} NBMOS, 268nm.
b: λ_{max} NBMOSuccn, 276nm.
d: λ_{max} OBr^-, 334nm.
e: rapid decompostion.

Since OBr^- is the reactive species that is of major interest in the decontamination process, these estimations of OBr^- production in various buffer solutions provide valid information about the reactivity of these N-brominating agents at the respective pHs.

Decontamination of mustard reagent by aqueous N-brominating agent.

The aqueous bleach[38] and N-chloramines[34] react in a manner more like SN_2 by attacking, initially, the sulfur atom of the mustard agent and converting it to a sulfoxide (S→O). The sulfoxides, more water soluble than mustard agents, will undergo further hydrolysis to give, ultimately, a complete oxidative cleavage of the mustard agent.

Higuchi et al.[114(a)] proposed a scheme (Eqn 2-10) to account for the oxidation of chloro mustard in basic and mildly acidic conditions. Since detailed studies on reaction of N-halo compounds with mustard compounds has not yet been reported in the literature, some analogy can be obtained from the reactions of chloramine-T with sulfur compounds reported in the literature.[44,114] These reactions are believed to follow second order process.[114(c)]



The reaction of aqueous N-brominating agent 47 with chloroethyl ethylsulfide (half mustard) was carried out at a molar ratio of 1:1 ($1.0x10^{-3}$ M) in pHs 9.3 and 11.0. The concentration of unreacted mustard in the reaction was analyzed on a G.C with a flame ionization detector every minute interval from the time of mixing. The results show no detectable (sensitivity limit $>5x10^{-5}$ M) mustard after a minute of reaction at both the pHs. This indicated that about 99% of mustard was destroyed before first sample could have been taken after the addition of 47 to the mustard in buffer solution. Admittedly, the sensitivity of the ionization detector was often hampered by a "sulfur" residue deposition on the flame detector tips and the tip was replaced several times during the course of the study, which prevented more quantitative results to calculate $t_{1/2}$ of this reaction.

Overall, the rapid reaction of 47 with chloroethyl ethyl sulfide can be compared with that of compound 43 ($t_{1/2} \approx 43$ sec) at pH 9.3. It appears according to Higuchi's scheme that the reactive species involved in both pHs (9.3 and 11.0) is the OBr^- ion. However, the pk_a of hypobromos acid (HOBr) is 8.5, and hence the decontamination at pH 9.3 would have partial contribution from the reaction of free acid (HOBr) with mustard. Higuchi et al.[114] reported that the reaction of hypobromus acid at neutral conditions (pH 8-7) is more rapid than basic conditions. It is noteworthy that at alkaline conditions overall hydrolysis of chloroethyl ethyl sulfide will be a combination of chlorine displacement by OH^- ion and nucleophilic attack at the sulfur by OBr^-.[46]

Decontamination (hydrolysis) of the organophosphate (Paraoxon)) by aqueous N-brominating agents

The continuous main objective of the Universal Decontamination project is to significantly accelerate the hydrolytic cleavage of various phosphorus ester type chemical agents. This involve $SN_2(P)$ displacement of the leaving group (e.g., fluorine) by the hyphochlorite anion (OCl^-) and subsequent hydrolytic cleavage of the corresponding oxychloro phosphate.

Bodor et al.[38(b)] have suggested that the reaction of 3-bromo-4,4-dimethyl-2-oxazolidinone (43) with GD and VX (Figure 1-1) at pH 10 goes through nucleophilic attack of OBr^- ion, and hence corresponds to the conversion of the N-bromo compound to its free amine and OBr^- ion reaction. Thus, the steady and extensive formation of active oxidizing species (OBr^-) from a bromine source would provide the basis for an aqueous decontaminating system.

Compounds 47 and 49 were investigated for their ability to hydrolyze organophosphates in aqueous medium using Paraoxon as the model organophosphate. Hydrolysis of Paraoxon was carried out at pseudo first order condition with various concentrations of N-bromo compounds at different pH; the kinetic data are illustrated in Table 2-19 and 2-20. The reactions were carried out in alkaline medium (pH 8.0 to 11.5) in which the reaction of N-bromo compound with the aqueous medium would provide ambient concentration of OBr^- species.

Hydrolysis of Paraoxon by 47 and 49 increased with increase in pH, which corresponds (after correcting for buffer hydrolysis) to the increase in concentration of OBr^- ion (Table 2-19) with the increase of pH. Both 47 and 49 show optimum rate enhancement of 6.0 fold and 4.2 fold respectively between pH 10.6-10.9. In general, compound 47 appears to be more effective than 49 in the hydrolysis of Paraoxon. Where the pseudo first-order rate constant for the reaction of 47 with Paraoxon is almost 4 times more than that of 49. The low reactivity of 49 at high pH can be explained by the instability of 49 at a high concentration of OBr^- which is also evident from the stability profile.

Table 2-19: Hydrolysis of Paraoxon[a] by NBMOS (47) as a function of pH at 30°C.

pH[b]	47, M 10^3x	$t_{1/2}$ [c] min	K_{obs} 10^3xmin^{-1}	K_{obs}/K_{buff}
8.03	1.76	24.9 dys	0.019	---
9.97	1.76	841	0.82	2.4
10.56	5.27	187.4	3.70	6.0
10.56	1.78	264.8	2.62	
10.70	5.27	153.2	4.25	3.8
11.20	5.27	46.1	15.02	5.41

a: buffer, 0.2M.
b: Paraoxon $5.5x10^{-5}$M.
c: r = 0.998.

Table 2-20: Hydrolysis of Paraoxon[a] by NBMOSuccn (49) as a function of pH at 30°C.

pH[b]	49, M 10^3x	$t_{1/2}$[c] min	K_{obs} 10^3xmin^{-1}	K_{obs}/K_{buff}
9.4	5.04	1382	0.50	---
10.91	5.04	108.4(806)[d]	6.4	2.6
10.91	2.5	190(13.5hrs)[d]	3.6	4.2
11.43	4.70	105	6.5	1.45[e]

a: Paraoxon, $5.5x10^{-5}$M.
b: buffer, 0.2M.
c: r =0.998.
d: at 25 °C.
e: rapid decomposition.

To investigate the kinetic process the reaction between 47 and Paraoxon was carried out at pH 11.20 at various concentration of 47 and the kinetic data are summarized in Table 2-21. The pH 11.20 was chosen for this reaction because more than 70% of [47] would be converted to OBr^- species at this pH. The plot (Figure 2-35) of pseudo first-order rate constant against concentration of 47 showed a linear corelation and the intercept ($2.6x10^{-3}$ m^{-1}) of the plot was in close agreement with rate costant (k_b=$2.91x10^{-3}$ m^{-1}) of OH^- hydrolysis of Paraoxon at pH 11.20. The apparent second-order rate constant calculated (from the slope) for this process is 2.16 M^{-1} min^{-1} (r^-=0.98).

Table 2-21: Hydrolysis of Paraoxon[a] as the function of the concentration of NBMOS (47) in pH 11.17[b] at 35°C.

NBMOS (47), M	$t_{1/2}$, min[c]	10^{-3}xK_{obs}, min^{-1}
$1.51x10^{-3}$	101.5	6.83
$3.08x10^{-3}$	88.6	7.81
$4.54x10^{-3}$	57.8	12.0
$6.17x10^{-3}$	40.70	17.31
$9.25x10^{-3}$	31.22	22.3
buffer	238.1	2.91

a: Paraoxon, $3.5x10^{-5}$M
b: buffer, μ = 0.6
c: r = 0.998

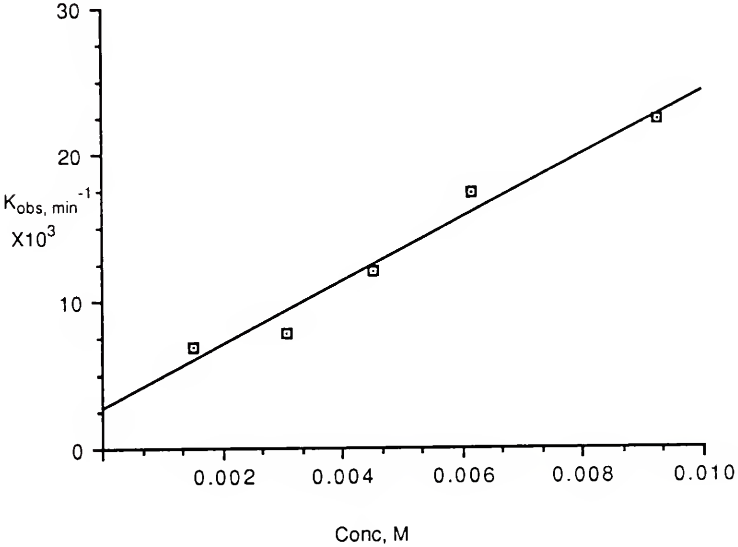


Figure 2-35: Hydrolysis of Paraoxon at various concentration of NBMOS (47) in pH 11.2 at 35°C.

Overall, the rection is a second order process with respect to OBr^- and Paraoxon concentration. The aqueous stability of the bromine source parallels its hydrolytic potency. It can be concluded that N-bromo compounds 47 and 49 provide a basis for water soluble, "soft" and stable source of OBr^- ion for the organophosphates decontamination.

CHAPTER III
EXPERIMENTAL

Materials

All solvents were routinely stored over 4 Å molecular sieves. Solvents used in reactions were either distilled using standard methods or were commercially available purest grade ("sure seal":Aldrich). All reactions requiring anaerobic conditions or moisture free conditions were carried out under positive argon atmosphere or with an attachment of dry tube containing "Drierite" ($CaSO_4$).

Triethylamine (Et_3N) reagent grade was distilled over sodium hydroxide. Thionyl chloride ($SOCl_2$), purest grade was purchased from Fluka Chemical Company. Ethanol of the purest available grade (Florida Distillers) was used. Diethyl ether used in synthesis was obtained from freshly opened cans. Chloroform (ethanol free) was dry distilled over $CaSO_4$. n-Butyllithium (n-BuLi) and lithium diisopropylamide (LDA) were purchased as standard solutions from Aldrich Chemical Company and stored at 0°C. Catalysts 5% Pd/C (Aldrich) and 2% $Pd/SrCO_3$ were purchased. 1,3-Bis(diethylamino)-2-propanol (Aldrich), 2-diethylaminoethanol (Aldrich), Eschenmosers salt (Aldrich), isomers of pyridinealdoxime (Aldrich), O-benzyl hydroxylamine (Aldrich), uracanoic acid (Aldrich), Paraoxon (Aldrich) 4-nitrophenyl dichlorophosphate (Aldrich) bis (4-nitrophenyl) phosphate, (free acid, Sigma), phenyl 4-nitrophenyl phosphonate (free acid, Sigma), alkalinephosphatase (Type III-S, Sigma) and bromoacetic acid anhydride (Lancaster) were purchased from commercial sources and used without purification.

Generally, all synthetic intermediates were stored at room temperature. The brominated products were stored under dark in the desicator. Final quaternary products were stored in dessicators at room temperature.

Due to the extreme toxicity associated with organophosphates, reactions were carried out in a well ventilated hood. All transfers of reagents were made under the hood using butyl rubber gloves and gas masks (Willson 6100). All glassware and reaction solutions were detoxified with excess of 2% aqueous sodium hydroxide and allowed to stand for 24h before discarding. The final and intermediate organophosphorus products were stored in tightly capped (sealed) containers at +5°C.

Methods

All melting points reported are uncorrected. Thin layer chromatography (TLC) was carried out using Merck Kiesel gel F_{254} aluminum plates. Preparative TLC was carried out using Merck Silicagel 60 F_{254} coated (0.25-0.50 mm) glass plates. Column chromatography was carried out using Davisil Silicagel (100-200 mesh). Infrared spectra were recorded on a Perkin-Elmer 283B or an FT-IR Perkin-Elmer 1600 spectrometer. ^{1}H NMR spectra were recorded on either a Varian EM-300L (60 MHz), or EM-390 (90 MHz), or Varian XL200 (200 MHz) or on a Varian XL (300 MHz), or GE 300 (300 MHz). ^{13}C NMR spectra were recorded on a Nicolet NT300 (75.45 MHz), or Varian XL300 (75.0 MHz) spectrometers. Mass spectra was recorded on a Kratos MS 80RFA spectrometer, either by

EI or Fast Atom Bombardment (FAB) methods. Ultraviolet spectra were recorded on a Hewlett Packard model 8451A equipped with a thermostated cell compartment. Fluorescence spectra were measured on a Kontron Spectrfluremeter SFM23/B. Elemental analysis (C, H, and N) were performed at Atlantic Micro Lab, Atlanta. A gas chromatography Gow-Mac Model 740P with flame ionization detector and Perkin Elmer LC1-100 integrator were employed for detection of mustard agent. An Orion Research Digital Ionalyz pH meter and pH indicator strip pH 7.5-9.5 were used for pH measurements.

Chromatographic Solvent Systems

(A) chloroform/methanol 9:1

(B) chloroform/methanol 4:1

(C) ethyl ether/petroleum ether (40-60°C), 1:1

(D) ethyl acetate/methylene chloride 1:1

(E) methylene chloride/methanol/acetic acid 4:1:0.1

Compounds

Hydroxamic Acids

Benzyl uracanoate (61)[60]

In a (100 mL) flask equipped with a drying tube, a mixture of uracanoic acid (20 g, 0.145 mol) and thionyl chloride (25 mL) was stirred for 1h at room temperature. The excess thionyl chloride was removed under reduced pressure. Benzyl alcohol (50 mL, 0.48 mol) was added to the above mixture and the reaction mixture was stirred at room temperature for 24 h. The crude benzyl ether was collected by filtration, suspended in water, and then a 50% sodium hydroxide solution was added until the mixture was basic. After the solution was stirred for 1h, product precipitated. The precipitate was washed with water and dried to give 61 (14.41 g, 44% yield): mp 197-199°C. The structure was confirmed by ^{1}H NMR.

^{1}H NMR (90 MHz, $CDCl_3$) δ7.75 (1H, d of d), 7.5-7.35 (m, 6H), 7.3 (s, 1H), 6.55 (d, J=12Hz, 1H) 5.3 (S, 2H) IR (KBr): 3050 (W), 3010 (W), 1700 (C=O), 1620 cm^{-}.

3-(1(N)-Methyl-4-imidazolyl)propenoyl benzyl ester (62)

Benzyl uracanoate (5.0 g, 0.022 mol) in DMF (5 mL) was added to a suspension of 60% sodium hydride (0.97 g, 0.024 mol) in DMF (10 mL) over 20-30 min at room temperature under argon. After evolution of hydrogen gas has ceased the reaction was warmed at 60-70°C for 1.5h, and then allowed to cool to 10°C. Methyl iodide (3.28 g, 0.023 mol) in DMF (mL) was added over 20 min at 10°C and stirred at room temperature for 3h. A few drops of methanol was added to the reaction medium and the reaction mixture was evaporated to dryness. Product was extracted with chloroform and small amount of ethyl ether was added to the chloroform layer to induce crystallization. The crystals were collected and dried to give 1 (N)-methyl-4-imidazole propanoyl benzyl ester (30% yield): mp 124-126°C.

^{1}H NMR (60 MHz, $CDCl_3$) δ 7.8 (s, 1H), 7.5-7.1 (M, 6H), 6.96 (s, 1H), 6.5 (d, J=12 Hz, 1H), 5.2 (S 2H), 3.6 (S, 3H).

3-[4-Imidazolyl]propanoic acid (63)

Benzyl uracanoate (5.0 g, 0.022 mol) in 50% methanol-water solution was hydrogenated at 50 PSI for 12h over 5% Pd/C catalyst. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure to give 63 (87.6% yield (Lit.[60] 15%)).

^{1}H NMR (60 MHz, D_2O)) δ 8.5 (s, 1H), 7.10 (s, 1H), 2.7 (dd, 2H, 2.4 (dd, 2H)

3-[1(N)-Methyl-4-Imidazolyl]propanoic acid (64)

Benzyl 3-(N)-methylurocanoate (1 g, 7.2 mmol) was hydrogenated using the same procedure as described used for benzyl uracanoate (above) to give the free acid. (0.71 g, 61% yield)

^{1}H NMR (60 MHz, D_2O) δ 8.7 (s, 1H), 7.20 (s, 1H), 4.00(s, 3H), 3.0 (d of d, 2H), 2.8 (d of d, 2H).

Benzyl benzohydroxamate (56)[116]

To a solution of benzohydroxamic acid (25.0 g, 0.182 mol) in dry ethanol (91 mH) was added potassium hydroxide (1.50 equivalents, 85%, 18.02 g) in methanol (32 mL) at 30-40°C over 30 min with stirring. The temperature was controlled by occasional cooling in an ice bath. After all alkali had been added, the mixture was allowed to stand in an ice-bath for 5-10 min to ensure complete precipitation of potassium chloride. The mixture was filtered and allowed to stand at room temperature overnight. The solvent was evaporated to half original volume in order to induce crystal formation. The crystals were collected , washed with ethanol and dried under air to give the potassium salt of benzohydroxamic acid (19.84 g).

To a solution of benzohydroxamate potassium salt (13.7 g, 0.06 mol), potassium carbonate (3 g) in methanol (50 mL) was added benzyl bromide (12,83 g, 0.075 mol) and water (30 mL). This mixture was stirred for 24h and methanol was removed, and the residue was acidified with 12M hydrochloric acid. The crude product was then taken up in 6M sodium hydroxide (4 x 100 mL). The combined sodium hydroxide layer was acidified with 12M hydrochloric acid and the aqueous solution was extracted with chloroform (4 x 50 mL). Chloroform was stripped off under reduced pressure to give a solid and recrystallized from ethyl ether/petroleum ether (40-60°C) to give benzyl benzohydroxamate (8.2 g, 60% yield): mp 103-104°C (lit. 104-105°C).

^{1}H NMR (60 MHz, $CDCl_3$) δ 8.9 (br), 7.8-7.3 (m, 10H), 5.2 (s, 2H).

O-Benzyl-N-(β-diethylaminoethyl)benzohydroxamate (57)

Benzyl benzohydroxamate (1.52 g, 7.0 mmol) in dry DMF was added dropwise to 60% sodium hydride (6 mmol) suspended in DMF (25 mL) under argon. After all the hydrogen gas had evolved, the reaction mixture was stirred for 1h at 40-50°C. A solution of chloroethyl diethylamine (free amine was obtained from neutralization of the hydrochloride salt with 0.1 N sodium hydroxide at 0°C and extraction with benzene, 5.0 mL) was added at once, and the mixture was stirred for 12h at 60-70°C. The reaction mixture was filtered and neutralized with 1M hydrochloric acid to pH 5-6, and the solvent was removed in vacuo to give 57 (47% yield).

^{1}H NMR (60 MHz, $CDCl_3$) δ 7.7-7.4 (m, 10H), 5.2 (d.s, 2H), 3.4-2.8 (m, 8H), 1.15 (t, 6H); IR (Nujol); HCl salt: 3500-3200 (br, NH), 1715 (m, C=O), 1660 & 1610 (w) cm^{-1}.

O-Benzyl-N-[3-(4-imidazolyl)propio]benzohydroxamate (attempted).

To 3-(4-imidazolyl)propionic acid (0.46 g, 2 mmol) was added thionyl chloride (2 mL) and stirred at room temperature for 1h under dry conditions. The excess of thionyl chloride was removed under vacuum to give the corresponding acyl chloride. This was used immediately in the next reaction.

To 60% sodium hydride (0.21 g, 5 mmol) in DMF (3 mL) was added benzyl benzohydroxamate (0.93 g, 4.1 mmol) in DMF (6 mL) over 30 min at room temperature under argon. After evolution of hydrogen was almost completed the mixture was stirred at 50°C for 1h. After bringing the reaction mixture to room temperature, 4-imidazole propanoic acid chloride (2 mmol) in DMF (6 mL) was added over 1h at 60°C. The reaction mixture was stirred further at 60-70°C for 2h. After cooling to room temperature, a small amount of methanol was added and the reaction mixture was filtered. The filtrate was adjusted to pH 4-5 with conc. hydrochloric acid, and the solvent was evaporated *in vacuo*. 1M Hydrochloric acid (20 mL) was added to the oily residue and the excess of benzyl benzohydroxamate was suspended as precipitate. The solution was repeatedly washed with benzene, its pH was adjusted to 3-4 and evaporated to dryness. The solid residue was extracted with absolute ethanol and evaporated to dryness to give a white-green solid. The ^{1}H NMR of this solid gave complicated patterns.

Hydrolysis of this solid to its debenzoyl compound was attempted by dissolving in ethanol/hydrochloric acid. The product isolated from the aqueous layer could not be characterized by ^{1}H NMR.

N-(β-Diethylaminoethyl)-O-benzylhydroxylamine (58)

(i) A direct method. O-Benzylhydroxylamine hydrochloride (25 g) was dissolved in water (100 mL) and benzene (100 mL) was added to this solution. The mixture was neutralized with sodium hydroxide (0.1 M) at +5°C using phenolphthalein as the indicator. The benzene layer was

washed with water (2 x 25 mL) and the solvent was stripped off to give O-benzylhydroxylamine (free amine).

O-Benzylhydroxylamine (13.43 g, 0.109 mol), chloroethyl diethylamine hydrochloride (19.69 g, 0.114 mol) and dry triethylamine (23.1 g, 0.23 mmol) in dry chloroform (75 mL) was stirred under reflux and argon atmosphere overnight. The insoluble portion was removed by filtration and washed with excess (100 mL) of chloroform. The filtrate was washed with 5% sodium bicarbonate (2 x 50 mL), water (2 x 25 mL) and dried (Na_2SO_4). The solvent was evaporated to give a oil and this oil was distilled (bulb to bulb) to give the product 56 (8.7 g, 36% yield): bp 52-57°C/≈ 0.2 mm Hg. Also a high boiling fraction was isolated and was shown to be the disubstituted product by ^1H NMR.

Data for the low boiling product (50-55°C / ≈ 0.02 mm Hg) 58: ^1H NMR (90 MHz, $CDCl_3$) δ 7.5 (s, Ph, 5H), 4.9 (s, 2H, 3.3 (m, 2H), 2.7 (q, 6H), 1.3 (t, 6H); MS (EI) 243 (M^+-1).

Data for the highboiling fraction (70-80°C/≈0.2 mm Hg) 59: 1H NMR (90 MHz, $CDCl_3$) δ 7.5 (s, Ph, 5H), 4.9 (s, 2H), 3.3 (m, 4H), 2.7 (m, 12H), 1.3 (t, 12H).

(ii) Via hydrolysis of O-benzyl-N-(β-diethylaminoethyl) benzohydroxamate (attempted). To a solution of 57 (1 g) in ethanol (20 mL) conc. hydrochloric acid (3 mL) was added dropwise and stirred for 30 min at 40-50°C. To this reaction mixture was added water (30 mL) and the aqueous layer was washed with benzene in order to remove benzoic acid and other hydrolyzed products. The aqueous layer was evaporated to give a crude white salt mixture. ^1H NMR and IR spectral data of this solid indicated cleavage of benzyl group instead of benzoyl group had occured. TLC of the crude product showed the presence of two products. Column chromatographic separations of this mixture gave two products. The spectral data of the major product did not correspond to the expected product, but rather corresponded to N-diethylaminoethyl benzohydroxamate. The minor product was found to be the salt of diethylamine.

Data for the major product; ^{1}H NMR (60 MHz, DMSO-d_6) δ 8.3-7.3 (m, 5H), 4.4 (t, 2H), 3.0-2.6 (m, $\approx$ 6H), 1.05 (t, 6H); IR (Nujol): 3550-3250 (br), 1715 (m) 1650 & 1500 (w), 1400 cm^{-1}.

The above hydrolysis was carried out under conditions such as (a) 6 M HCl (2 mL)/THF (6 mL), (b) conc. HCl/THF (6 mL)/EtOH (1 mL), (c) conc. HCl/EtOH (10 mL)/ H_2O (3 mL)/$CHCl_3$ (1 mL) at room temperature or 60-70°C.

Product(s) obtained did not agree with the expected product. Under strong hydrolytic conditions complete hydrolysis of the starting material was observed. Milder conditions yielded N-diethylaminoethyl-O-benzoyl hydroxamate (benzyl cleaved product), and in some cases HCl salt of the starting material was obtained.

N-(β-Diethylaminoethyl)hydroxylamine: a direct approach (attempted)

To a mixture of hydroxylamine hydrochloride (5.0 g, 0.072 mol) and sodium carbonate (0.144 mol) in methanol (20 mL) was added chloroethyl diethylamine (9.76 g, 0.072 mol) and refluxed for 2h. Reaction was monitored by TLC (solvent system E). The methanol layer was evaporated to give a white solid, and the residue was suspended in water and the pH was adjusted to 10 with 50% sodium hydroxide. The aqueous layer was extracted with chloroform, dried (Na_2SO_4) and the solvent was stripped off to give faint yellow oil. IR spectrum of the oil indicative of free hydroxyl group. However, ^{1}H NMR spectrum failed to agree with the structure of expected product.

The above synthesis was repeated with sodium hydroxide in water (5 mL) and stirred for 3 days at room temperature. The product isolated using a similar work-up, showed reasonable IR and ^{1}H NMR spectra for the expected product. However, mass spectrum of the product showed a pattern for higher mass than expected.

^{1}H NMR (90 MHz, $CDCl_3$) δ 7.5 (br), 2.7-2.3 (m, n8H), 1.0 (t, n6H); IR (neat) 3400 (br, OH), 3100-2900 (s) 99 (50), 86 (100) cm^{-1}.

Benzyl N-(β-dimethylaminoethyl)-3-(4-imidazolyl)propenohydroxamate (65)

To uracanoic acid (3.54 g, 25.6 mmol) was added thionyl chloride (Fluka, 14.0 g, 8.2 mL) at room temperature and the reaction flask was warmed to activate the reaction. The reaction was stirred at room temperature for 1h and then refluxed with dry benzene (20 mL) for 1h under argon atmosphere. The solvent and excess thionyl chloride were evaporated carefully on a rotor vap under dry conditions and the resulting solids were dried under vacuum overnight.

To a stirred solution of 58 (5.5 g, 25 mmol) in dry triethylamine (25.0 g, 0.25 mol) and ethanol free dry chloroform (25 mL) was added urocanoic acid chloride in chloroform (20 mL) via a syringe (with wide bore needle) over 5 to 10 min. The reaction was initially stirred at room temperature for 1-2h and then heated at gentle reflux over 12h. The reaction mixture was brought to pH 11 by treating with 50% NaOH. The products were extracted with chloroform (3 x 100 mL), washed several times with water until a clear organic layer resulted and dried (Na_2

SO_4). Solvent was stripped off from the organic layer to give a faint color oil. This oil was suspended in petroleum ether overnight and carefully chromatographed through silica gel (8" column, 100-200 mesh) eluting with solvent system-1 to give 3 major components (R_f 0.60, R_f 0.34, R_f 0.18). The middle component (R_f 0.34) was found to be the desired product (11% yield). High R_f component gave spectral data mainly for the unreacted starting hydroxylamine 58. Low R_f (0.18) was spectrally evident for structure 66.

Data for the product (R_f 0.34; solvent B): ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.5 (d, J≈15 Hz, 1H), 7.38 (s, Ph, 5H), 6.90 (d, J≈15 Hz, 1H), 4.95 (s, CH_2 Ph, 2H), 3.77 (t, J=8 Hz, 2H), 2.75 (t, J=8 Hz, 2H), 2.50 (q, 4H), 1.00 (t, 6H). ^{13}C NMR (74.75 MHz, $CDCl_3$) δ 167.9 (C=O), 137.1, 134.55, 134.0, 129.20 (2 x C), 128.86, 128.64 (2 x C), 113.16 (2 x C), 49.18, 46.97 (2 x C), 44.52, 11,32 (2 x C), MS (EI) m/e 343 (M^+1), 342 (M^+, 35), 121 (18), 107 (75), 86 (100).

Anal. Calcd. for $C_{19}H_{26}N_4O_2$. 0.5 H_2O: C, 64.94; H, 8.03, N, 15.94. Found: C, 64.87; H, 7.72; N, 15.68.

Data for the component (R_f 0.18; solvent B): ^1H NMR (90 MHz, $CDCl_3$) δ 7.6 (S, imidazole, 1H), 7.4 (s, Ph, 5H), 7.35 (s, Ph,5H), 6.9 (s, imidazole, 1H), 4.9 (s, 2H), 4.80 (s, 2H), 3.7 (t, 2H), 3.15 (s.d, 2H), 2.80-2.40 (m, 10H), 0.90 (S.t, 12H); MS (EI) m/e 565 (M^+1), 475 (5), 343 (40), 223 (15), 86 (100).

Data for the component (R_f 0.60; solvent B): MS (EI) m/e 321 (15), 283 (30), 221 (M^+-1, 40), 167 (52), 121 (90), 86 (100).

O-Bnzyl-N-(β-dodecyldimethylammonium bromide)ethyl-3-[3(N)-dodecyl-4-imidazolylium hydrobromide]propenohydroxamate (67)

Free amine 65 (0.2 g, 0.58 mmol) was gently refluxed with bromododcane (1 mL) in chloroform (16 mL)/acetonitrile (4 mL) for 4 days. The reaction mixture was evaporated, and the residual oil was dissolved in methanol and the insoluble salts were removed by filtration. The filtrate was evaporated to dryness and the resulting thick oil was suspended in ethyl ether to give a hygroscopic residue. This residue was dried under vacuum to give semi solid 67 (0.31 g, 63% yield).

^{1}H NMR (300 MHz, CD_3CN/CD_3OD) δ 8.7 (s, 1H), 7.7 (split.d,1H), 7.5-7.4 (m, 5H), 7.10 (d, J≈12 Hz, 1H), 5.03 (s, $\underline{CH_2}$ Ph, 2H), 4.3-3.90 (m, 6H), 3.5-3.10 (m, 8H), 1.8-1.10 (m, 46H), 0.9 (t, 6H). MS (FAB) m/e 680 (M^+), 571 (40), 500 (70), 332 (100), 289 (95).

Anal. Calcd. for $C_{43}H_{76}B_4O_2Br_2$. 0.5 H_2O: C, 60.77; H, 9.13; N, 6.59. Found: C, 60.91; H, 9.57; N, 6.57.

N-(β-dodecyldimethylammonium bromide)ethyl-3-[(3(N)-dodecyl-4-imidazolylium hydrobromide]propiohydroxamic acid (14)

(i) Hydrogenation in EtOH/H_2O. O-Benzyl hydroxamate 67 (0.20 g, 0.026 mmol) was hydrogenated with 5% Pd/C (214 mg) in ethanol (6 mL)/water (8 mL) at 30 PSI over 16h. A rapid hydrogen uptake was observed over 30 min. The catalyst was removed by filtration and the solvent was stripped off from the filtrate to give an oily residue. The inorganic salts were precipitated by dissolving the oil in acetonitrile (10 mL). The filtrate was evaporated and dried under vacuum overnight to give 14 (70 mg, 39%).

^{1}H NMR (300 MHz, CD_3CN/CD_3OD) δ 8.8 (s, 1H) 7.5 (s, 1H), 4.3-4.0 (m, 4H), 3.7-2.9 (m, 12H), 1.9-1.0 (m, 46H), 0.98 (t, 6H). MS (FAB) m/e 591 (M^+-1, 90), 532 (100), 433 (85), 291 (98), 137 (95). λ_{max} pH 9.4: 240 nm

Anal. ($C_{36}H_{70}N_4O_2Br_2$. 2.5 H_2O) H, N; C: Calcd, 70.65; Found, 70.18.

(ii) Hydrogenation in EtOH. O-Benzyl hydroxamate 67 was hydrogenated in dry ethanol under conditions described in method (i) (as above). No debenzylated product(s) was observed over 2 days under this condition.

O-Benzyl-N-(β-methyldiethylammonium iodide)ethyl-3-[3(N)-methyl-4-imidazolylium hydrobromide]propeniohydroxamate (68)

Free amine 65 (0.26 g, 0.76 mmol) in chloroform (10 mL)/acetonitrile (5 mL) was stirred with methyl iodide (1 mL) initially at +5°C for 3h and then at 40°C for 2 days in a pressure bottle. The solvent was evaporated and the oily residue was suspended in ethyl ether overnight. The crude solid was suspended in chloroform, and the insoluble portion was separated and dried under vacuum to give a faint yellow solid (0.41 g, 86% yield).

^{1}H NMR (90 MHz, CD_3OD) δ 8.4 (s, 1H), 7.35 (s, 1H), 7.3-6.8 (m, 7H), 5.1 (s, $\underline{CH_2}$Ph, 2H), 4.3-3.9 (m, 2H), 3.7 (s, 3H), 3.5-3.10 (m, 6H), 3.0 (s, 3H), 1.15 (t, 6H); MS (FAB) m/e 284 (M^+-(N^+Et_2Me), 223 (10), 155 (55).

Anal. ($C_{21}H_{32}N_4O_2I_2$): H, N; C: Calcd, 54.51; Found, 55.32

N-(β-Methyldiethylammonium iodide)ethyl-3-[3(N)-methyl-4-imidazolylium hydrobromide]propiohydroxamic acid (15)

O-Benzyl hydroxamate 68 (0.10g, 0.16 mmol) was hydrogenated with 5% Pd/C (90 mg) at 30 PSI in ethanol (8 mL)/water (4 mL) over 6h. A rapid hydrogenation was observed over 30 min. The catalyst was filtered and the solvent was evaporated to dryness. The residue was dissolved in acetonitrile (5 mL), the insoluble portion was removed by filtration and the filtrated was evaporated to dryness under vacuum to give a hygroscopic (amorphous) solid (90% yield).

^{1}H NMR (300 MHz, D_2O/CD_3CN) δ8.6 (s, 1H), 7.34 (split s, 1H), 4.0 (t, 2H), 3.75 (double s, 3H), 3.61 (t, 2H), 3.34 (q, 6H), 3.05,2.90 (double s, 3H), 1.15 (t, 6H). MS (FAB) m/e 283 (M^+-HI), 269 (10), 251 (15), 196 (18), 180 (22), 155 (100); λ_{max} pH 9.4: 248 nm.

Anal. ($C_{14}H_{26}N_4O_2I_2 \cdot 2H_2O$) H, N; C: Cacld, 44.45; Found, 43.39.

Pyridinium Compounds

2-(O-Benzyloxyiminomethyl)pyridine (69)

To pyridine-2-aldoxime (10 g, 0.082 mol) in abs. ethanol (60 mL) was added sodium methoxide (5.0 g, 0.09 mol) in ethanol (50 mL) over 30 min at 60°C. The solution was heated at reflux for 1h. Benzyl bromide (14.2 g, 0.083 mol) in ethanol (50 mL) was added to the above solution over 5 min at 60°C and stirred for 24h at gentle reflux. The reaction mixture was evaporated to dryness and the residue was extracted with petroleum ether (40-60°C, 3 x 50 mL), washed with water (2 x 20 mL) and dried ($MgSO_4$). The solvent was stripped off to give a faint yellow oil and the oil was kept under vacuum. The final oil was distilled on a

Kugelrohr apparatus at 100-110°C/≈ 1 mm Hg to give product 63 (7.74 g, 48% yield)

^{1}H NMR (90 MHz, $CDCl_3$) δ 9.0 (m, 1H), 8.5 (s, 1H), 8.1-7.4 (m, 8H), 5.4 (s, OCH_2Ph, 2H).

1-Ethyoxycarbonylmethyl-2-(O-benzyloxyiminomethyl)pyridinium bromide (71(a))

Pyridine-2-aldoxime O-benzyl ether (1.65 g, 8.3 mmol) and bromoacetate (6.6 g) were stirred at room temperature for 7 days to give a faint brown solid. The solid was filtered off and recrystallized from acetone to give product 71(a) (1.45 g, 50% yield): mp 119-120°C.

^{1}H NMR (60 MHz, $CDCl_3$) δ 9.8 (m, 1H), 8.7 (s, CH=N, 1H) 8.6-8.0 (m, 3H), 7.4 (s, Ph, 5H), 6.3 (s, N^+-CH_2, 2H), 5.35 (s, CH_2 Ph, 2H), 4.25 (q, CH_2CH_3, 2H), 1.3 (t, CH_3, 3H).

Anal. Calcd. for $C_{17}H_{19}O_3N_2$: C, 53.84; H, 5.05; N, 7.39; Br, 21.07. Found: C, 53.64; H, 5.10; N, 7.34; Br, 21.00.

1-Ethyoxycarbonylmethyl-2-(hydroxyiminomethyl)pyridinium bromide (71(c))

Pyridine-2-aldoxime (1.5 g, 0.013 mol) and ethyl bromoacetate (7.6 g) were stirred at room temperature for 4 days. The resulting white suspension was evaporated to dryness under vacuum to give a white solid. The solid was recrystallized from chloroform/methanol to give 71(c) (1.4 g, 37% yield): mp 152-153°C.

^{1}H NMR (90 MHz, $CDCl_3/CD_3OD$) δ 9.1-90 (m, 1H), 8.5 (s, CH=N, 1H), 8.4-8.0 (m, 4H), 5.8 (s, N^+-CH_2, 2H), 4.3 (q, CH_2CH_3, 2H), 1.3 (t, CH_3, 3H).

Anal. Calcd. for $C_{10}H_{13}O_3N_2$: C, 41.54; H, 4.55; N, 9.67; Br, 27.64. Found: C, 41.61; H, 4.55; N, 9.66; Br, 27.50.

3-(O-Benzyloxyiminomethyl)pyridine (70)

Procedure was identical to the one used for synthesis of 69. Product was distilled (Kugelrohr) at 85-90°C/≈0.3 mm Hg (47% yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ8.9-8.6 (m, 2H), 8.4 (s, CH=N, 1H), 8.3-8.0 (m, 2H), 7.5 (s, Ph, 5H), 5.2 (s, 2H).

1-Carboxymethyl-2-(O-benzyloxyiminomethyl)pyridinium bromide (71(b))

(i) Reaction with iodoacetic acid. Pyridine (O-benzyl)2-aldoxime (7.0 g, 0.033 mol) and iodoacetic acid (9.16 g, 0.050 mol) were heated at reflux in acetonitrile for 2 days. The resulting solution was evaporated to give a thick brown oil. The oil was chromatographed on a silica gel (100-200 mesh) with solvent system B. The crude product was isolated from the middle fraction and recrystallized from ethyl acetate/methanol to give faint yellow crystals 71(b)(less than 5% yield).

^{1}H NMR (90 MHz, CD_3OD/D_2O) 9.0-8.8 (m, 1H), 8.5 (s, CH=N, 1H), 8.4-7.8 (m, 3H), 7.2 (5, Ph, 5H), 5.7 (s, N^+ CH_2Ph, 2H).

(ii) Reaction with bromoacetic acid (attempted). Pyridine (O-benzyl)-2-aldoxime (2.0 g, 9.3 mmol) and bromoacetic acid (1.3g, 9.6 mmol) in nitromethane (10 mL) were heated at reflux for 3 days. To the dark reaction mixture obtained was added ethyl ether. The precipitate was filtered and dried. The spectral pattern corresponded to a decarboxylated form of the expected product. ^{1}H NMR showed CH_3 peak at δ4.5 corresponding to methyl pyridinium salt and absence of methylene pyridinium peak around δ5.5.

1-Carboxymethyl-3-(O-benzyloxyiminomethyl)pyridinium bromide (72)

Pyridine (O-benzyl)-3-aldoxime (3.0 g, 0.014 mol) and bromoacetic acid (2.16 g, 0.016 mol) in THF (20 mL) were stirred in a pressure bottle at 40°C for 2 days. A white precipitate was filtered off and recrystallized from acetonitrile to give 72 (80% yield).

^{1}H NMR (90 MHz, $CD_3OD/CDCl_3$) δ 9.35 (s, 1H), 9.2 (dd, 1H), 7.5 (s, Ph, 5H), 5.7 (s, N^+-CH_2, 2H), 5.4 (s, CH_2Ph, 2H); MS (FAB) m/e 271 (M^+), 155 (100).

Anal. Calcd. for $C_{15}H_{15}N_2O_3Br$: C, 51.30; H, 4.31; N, 7.98; Br, 22.75. Found: C, 51.35; H, 4.35; N, 7.96; Br, 22.70.

1-Carboxymethyl-2-(hydroxyiminomethyl)pyridinium bromide (71(d)

A solution of bromoacetic acid (2.0 g, 14.4 mmol) and 2-pyridine aldoxime (1.76 g, 14.4 mmol) in dry ethanol (10 mL) was stirred at room temperature for 3 days. The precipitate was collected on filtration and recrystallized from ethanol to give 71(d) (25% yield). ^{1}H NMR of the product agreed with the assigned structure.

^{1}H NMR (90 MHz, D_2O) δ 8.7 (s.s, 1H), 8.5 (s, CH=N, 1H), 8.3-7.9 (m, 3H), 5.3 (s, N^+-CH_2, 2H); MS (EI) m/e 181 (M^+), 169 (10), 152 (27), 122 (100), 104 (30), 79 (45).

1,3-Bis(trimethylammonium)-2-propanol dibromide (79(a))

To 1,3-bis(dimethylamino)-2-propanol (5.0 g, 0.034 mol) in chloroform (5 mL) was added an excess of bromomethane (50 mL, 2.0 M in ethyl ether) and the mixture was stirred at 0°C overnight. The resulting white precipitate was filtered and recrystallized from methanol/acetonitrile to give 79(a) (32% yield).

^{1}H NMR (90 MHz, D_2O) δ 3.60-3.40 (m, 5H), 3.2 (s, 18H).

1,3-Bis(butyldimethylammonium)-2-propanol dibromide(79(b))

1,3-Bis(dimethylamino)-2-propanol (14.6 g, 0.146 mol) and bromobutane (41 g, 0.30 mol) in chloroform (50 mL) were heated at reflux overnight. The solvent was evaporated and the residual oil was suspended in ethyl ether to give a white solid. This crude solid was recrystallized from acetonitrile to give 79(b) as white crystals (63% yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ 6.5 (br, OH), 4.4-4.2 (m, 5H), 3.9-3.2 (m, 16H), 1.7 (dq, 4H), 1.35 (dq, 8H), 0.98 (t, 6H).

1,3-Bis(dodecyldimethylammonium)-2-propanol dibromide (79(c))

To 1,3-bis(dimethylamino)-2-propanol (5.0 g, 0.034 mmol) in ethanol (50 mL) was added bromododecane (16.8 g, 0.15 mmol) and the solution was heated at reflux. The solvent was evaporated to give a thick oily residue which was then titrated in ethyl ether to give white solids. The solids were filtered and recrystallized (charcoal) from acetonitrile-ethyl ether to give 79(c) (60% yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ 4.2-4.05 (m, 5H), 3.6-3.10 (m, 16H), 1.6-1.05 (m, 40H), 0.95 (t, 6H).

Anal. Calcd. for $C_{29}H_{62}N_2O_1Br_2.H_2O$: C, 55.06; N, 10.80; N, 4.20. Found: C, 55.15; H, 10.63; N, 4.07.

2-(β-dodecyldiethylammonium)ethanol bromide (79(d))

Diethylamino ethanol (3 g, 0.012 mmol) and excess of bromododecane in ethanol were refluxed over 3 days. Charcoal was added to the reaction mixture and the filtrate was evaporated to dryness to give a thick oily residue. The residue was suspended in ether to give solids, and the solids were recrystallized from acetonitrile-ethyl acetate to give white flaky crystals of 79(d) (46% yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ 6.0 (br, OH), 4.15-3.4 (m, 10H), 1.9-1.05 (m, 26H), 0.98 (t, 3H).

1,3-Bis(butyldimethylammonium)-2-propyl bromoacetate dibromide (80(b))

(i) Use of neat bromoacetyl bromide. Bis (1,3-dimethylbutyl amino)-2-propanol dibromide (1.0 g, 1.59 mmol) and bromoacetyl bromide (1 mL) was stirred under anhydrous conditions for 4 days at 50°C. The excess bromoacetyl bromide was evaporated under high vacuum at 60°C. The oily residue was washed many times with diethyl ether, the residue was dissolved in acetone (15 mL) and treated with solid sodium carbonate (anhydrous) for 30 to 45 min. The resulting clear solution after filtration was evaporated to dryness to give a white hygroscopic solid (1.20 g, 62 % yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ 5.0 (s, CH-O, 1H), 4.2 (s, $BrCH_2$, 2H), 3.8-3.0 (m, 15H), 2.8 (dt, CH_2, 2H), 1.5 (dt, 4H, CH_2), 1.0 (t, CH_3, 3H). MS (FAB) m/e 383 (M^{2+}), 323 (60), 280 (45), 158 (57), 102 (100%).

(ii) Use of Et_3N/bromoacetyl bromide (attempted). Above reaction was carried out in the presence of Et_3N in dry chloroform. Non-isolable product mixture resulted after work-up. Repeated attempts to obtain 80(b) via this method were not fruitful.

(iii) Use of Pyridine/bromoacetyl bromide (attempted). Method (i) was repeated in the presence of equal molar pyridine and using chloroform as solvent, at 45°C. The reaction mixture was warmed to 60°C for 3-4h. Pyridine and chloroform were stripped off to give dirty brown oil. The ^{1}H NMR of the oil accounted for a product mixture, no further purification of this mixture was attempted.

(iv) Use of Diisopropylethylamine/bromoacetyl bromide (attempted). Method (iii) was carried out using equimolar diisopropylethylamine as the base. Final oil accounted for a product mixture. Attempts to isolate the product by solvent extraction and chromatography were not successful.

1,3-Bis(dodecyldimethylammonium)-2-propyl bromoacetate dibromide (80(c))

(i) Use of neat bromoacetyl bromide. 1,3-Bis(dimethyl dodecyl ammonium)-2-propanol dibromide (1.0 g, 1.59 mmol) and bromoacetyl bromide (2 mL) were stirred at 40-45°C for 4 days under dry conditions. Excess bromoacetyl bromide was evaporated under high vacuum at 60°C. The resulting oily residue was washed several times with ethyl ether and allowed to stand on ether overnight. The resulting semi solid was dissolved in acetone and stirred with solid anhydrous Na_2CO_3 until the solution was clear. The solvent was stripped of to give hygroscopic solid 80(c) (83% yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ 4.9 (s, CH-O, 1H), 4.2 (s, Br CH_2, 2H), 3.9-3.3 (m, 20H), 2.9-1.25 (m, 40H), 0.98 (t, 6H); MS (FAB) m/e 687 (M^{2+} + $^{82}Br^-$), 685 (M^{2+} + $^{80}Br^-$), 605, (M^{2+}); IR (Nujol) 3250 (br, N^+<), 1720 (s, C=O) cm^{-1}.

(ii) Use of bromoacetyl bromide/base(s) (attempted). Method (i) was carried out at various conditions in the presence pyridine or Et_3N. A crude oil isolated at each trial shwed a complex mixture of products (^{1}H NMR).

2-(Dodecyldiethylammonium)ethyl bromoacetate bromide (81)

2-(Dodecyl diethyl)ammonium ethanol bromide (0.87 g, 2.4 mmol) and bromoacetyl bromide (1.5 mL) were stirred at 45°C for 3 days, under dry conditions. Excess bromoacetyl bromide was evaporated under high vacuum at 55-60°C, on a rotary evaporator. The oily residue was extracted with chloroform (50 mL), washed with saturated sodium carbonate solution (2 x 15 mL) and water (2 x 10 mL). The dry chloroform layer was stripped off under reduced pressure to give a faint yellow solid which was then dried under vacuum to give 81 (0.86 g, 73% yield). The product was found to be homogeneous by ^{1}H NMR and was carried on to the next reaction without further purification.

^{1}H NMR (90 MHz, $CDCl_3$) δ 4.30 (s, CH_2Br, 2H), 4.0 (m, N^+CH_2), 3.8-3.5 (m, 8H), 2.9-1.1 (m, 26H), 0.98 (t, 3H). MS (FAB) m/e 406 (M^+-1), 362 (35), 286 (85), 86 (100).

1,3-Bis(trimethylammonium)-2-propyl bromoacetate (80(a)) (Attempted)

(i) Via activated ester. 4-Nitrophenyl bromoacetate (1.0 g, 2.5 mmol) and 1,3-bis (trimethylamino)-2-propanol (0.28 g, 1.3 mmol) in toluene were stirred with imidazole (0.10 g, 1.4 mmol) at room temperature for 2 days. The reaction mixture was evaporated to dryness and the residue was washed with methylene chloride. The remaining residue was dried *in vacuo*. ^{1}H NMR of the final oil showed that imidazole, 4-nitrophenol and the desired product were present. Due to the low ratio of the product among other components it was decided not to isolate the compound from this mixture.

Repeated attempts under various solvents and reagent concentrations resulted in either incomplete reaction or formation of only a trace amount of product.

(ii) Via mixed anhydride. To a solution of bromoacetic acid (1.0 g, 7.2 mmol) in acetonitrile (10 mL) was added trifluoroacetic anhydride (3.0 mL) and stirred for 10 min. To this solution 1,3-bis(trimethylamino)-2-propanol was added and stirred at room temperature for 4 days under argon. The solvent was removed by vacuum and the residue was redissolved in acetonitrile. The insoluble paste was removed by filtration and the filtrate was evaporated to give a thick oil. The oil was stirred with anhydrous sodium carbonate in dry acetone to give a clear thick oil. The ^{1}H NMR of the product did not indicate the presence of the expected product.

(iii) Via bromoacetic anhydride. 1,3-Bis(trimethylamino)-2-propanol dibromide (1.94 g, 5.78 mmol) was dissolved in DMF (20 mL) and to this solution was added bromoacetic anhydride (2.0 g, 7.7 mmol) in DMF (5 mL) under argon. To this stirred solution was added DMAP (0.07 g) and the reaction was allowed to run for 4-5 days. The reaction mixture was evaporated to dryness and excess of bromoacetic anhydride was removed by washing with ethyl ether. The resulting syrup was dried under vacuum and the solids were isolated. The ^{1}H NMR of the solid corresponded to the unreacted amino alcohol.

The above reaction was carried out with 4-toluene sulfonic acid (catalytic amount) and gave similar results. Use of pyridine as the solvent or as the base resulted in quaternization of 2-bromo ester(s).

4-Nitrophenyl bromoacetate

To a solution of bromoacetyl bromide (7.2 g, 36 mmol) in dry chloroform (ethanol free, 5 mL) was added 4-nitrophenol (5.0 g, 36 mmol) and diisopropylamine (4.65 g, 36 mmol) at 0°C for 1 h. The reaction mixture was stirred further for 16 h at room temperature. The reaction mixture was poured onto ice and extracted with chloroform (3 x 35 mL). The organic layer was washed with 0.5% sodium hydroxide (2 x 50 mL), water (2 x 50 mL) and dried (Na_2SO_4). The solvent was stripped off and the solid was suspended in chloroform/ethyl ether. Insoluble portions were filtere off and the filtrated was evaporated to give a faint pink solid (61% yield). ^{1}H NMR spectrum agreed with the desired product.

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.01 (J_{AB}=7.5 Hz, 2H), 7.11 (J_{AB}=7.5 Hz, 2H), δ 3.9 (s, $BrCH_2$, 2H).

Dodecyl bromoacetate

Dry dodecanol (3 g, 16 mmol) and bromoacetyl bromide (3 mL) were stirred at 50°C for 2 days under dry conditions. The reaction mixture was evaporated under reduced pressure and the resulting oil was extracted with chloroform (2 x 25 mL), washed with sodium bicarbonate solution (2 x 15 mL) and dried (Na_2SO_4). The solvent was stripped off from the organic layer to give pure dodecyl bromoacetate (81% yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ 4.2 (t, OCH_2, 2H), 3.8 (s, $BrCH_2$, 2H), 1.9-1.0 (m, 20H), 1.0 (t, CH_3, 3H).

Nonyl bromoacetate

Dry nonanol (3.25 g, 18mmol) and bromoacetyl bromide (3 mL) were stirred at room temperature for 2-3 days under dry conditions. The excess of bromoacetyl bromide was stripped off under vacuum to give an oily residue. The residue was extracted with chloroform (2 x 25 mL), washed with sodium bicarbonate solution (2 x 15 mL), washed with water (2 x 10 mL) and dried (Na_2SO_4). The organic layer was evaporated under vacuum to give pure nonyl bromoacetate (85% yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ 4.2 (t, OCH_2, 2H), 3.85 (s, $BrCH_2$, 2H), 1.85-1.10 (m, 14H), 1.0 (t, CH_3, 3H).

1-[1,3-Bis(dimethylamino)-2-propoxy]carbonylmethyl (O-benzyloxyimino)methylpyridinium bromide (73)

Transesterification (attempted). 1-Ethylacetate-2-[(O-benzyloximino)methyl] pyridinium bromide (2 g, 5.4 mmol) and excess of bis(1,3-dimethylamino)-2-propanol (1.5 mL) in chloroform (ethanol free) were warmed at 60°C for 2 days. A dark solid resulted on addition of ethylacetate and this residue was suspended in chloroform and filtered to give a dark brown solid. The ^{1}H NMR of this solid did not correspond to the expected product, but rather corresponded to the decarboxylated product (1-methyl)-2-(O-benzyloxyiminomethyl) pyridinium salt. ^{1}H NMR (60 MHz, D_2O) δ 9.5 (m, 1H), 8.4 (s, CH=N, 1H), 8.3-7.9 (m, 3H), 7.4 (s, 5H), 4.6 (S, N^+CH_3, $\approx$3H).

The above procedure was repeated numerous times at various conditions such as in the presence of catalysts (zinc chloride, p-toluene sulfonic acid, etc.), with various solvents; all such attempts gave similar results.

Esterification using DIC (attempted). To pyridinium-3-oxime acetic acid 72 (1.0 g, 2.5 mmol) in dry DMF (10 mL) was added diisopropylcarbodiimide (DIC, 0.63 g, 5.0 mmol) and the mixture was stirred for 10 min. To the above solution was added (bis(1,3-dimethyl amino)-2-propanol (5.0 mmol) in DMF (2 mL). Stirring was continued for 5-6h under argon. No formation of DIU (diisopropylurea) was observed even after prolonged reaction time (2 to 3 days). Reaction was stopped after 3 days and the solvent was removed from the reaction mixture. The crude residue showed a ^1H NMR spectrum for unreacted starting material.

Esterification using DCC/DMP (attempted). The pyridinium-3-oxime acetic acid 72 (1.0 g, 2.85 mmol) in acetonitrile (15 mL) was added to DCC ("Gold label":Aldrich, 0.088 g, 4.3 mmol) followed by addition of bis(1,3-dimethyl)-2-propanol (0.46 g, 3.14 mmol) in acetonitrile (5 mL) and stirred at room temperature, under positive argon atmosphere. The reaction was stopped after 2 days and the precipitated dicyclohexylurea (DCU) was removed by filtration. The filtrate was evaporated to give brown solid. The major component was isolated through a silica gel column (100-200 mesh) by eluting with solvent system-2. The isolated product showed ^1H NMR spectrum for the DCU adduct of the acid (76), but not the expected product. Mass spectrum of this product showed the molecular ion (477) corresponding to the mass of the DCU adduct.

^1H NMR (90 MHz, $CDCl_3$) δ 9.4 (s, 1H), 8.6 (d.d, 1H), 8.4 (s, CH=N, 1H), 8.0-7.7 (m, 2H), 7.4 (s, Ph, 5H), 6.0 (s, N^+CH_2, 2H), 5.3 (s, OCH_2Ph, 2H), 3.6 (m, 2H), 2.0-1.0 (m, 20H). MS (FAB) m/e 477 (M^+), 352 (32), 265 (80), 183 (100)

Above reaction was repeated with the more polar solvent, DMF, and the same adduct was obtained. Attempts to convert the adduct to the product by reacting with excess of Bis(1,3-dimethylamino)-2-propanol under various conditions were not successful.

1-[1,3-Bis(butyldimethylammonium)-2-propyoxy]carbonylmethyl-3-[(O-benzyloxyiminomethyl]pyridinium tribromide [(17(a)) via N-alkylation with bromoacylester]

The bromoacylester 84(b) (1.20 g, 1.53 mmol) and 3-pyridine O-benzylaldoxime (0.36 g, 1.7 mmol) in acetonitrile (10 mL) were stirred in a homemade pressure bottle at 40°C for 3 days, under argon. The resulting white precipitate was filtered and washed with fresh acetonitrile. The solids were suspended from methylene chloride/acetone to give 17(b) (0.60 g, 52%).

Solubility: CH_3Cl_3 (soluble), MeOH (soluble), CH_3CN (sparingly soluble).

^1H NMR (90 MHz, DMSO-d_6) δ 9.97 (s, 1H), 9.5 (sd, 1H), 9.0 (s.s, 1H), 8.65 (s, $\underline{CH}$=N, 1H), 7.5 (s, Ph, 5H), 6.05 (s, NCH_2, 2H), 4.5-3.0 (m, 15H), 1.9 (split s, (CH_2), 4H), 1.5 (split s, (CH_2), 4H), 1.05 (t, CH_3, 6H). MS (FAB) m/e 593 $(M^{2+}+{}^{82}Br^-)$, 590 $(M^{2+}+{}^{80}Br^-)$, 396 (10), 142 (75), 100 (100). IR (Nujol) 1735 (C=O(OR)), 1600 (m, C=N).

Anal. Calcd. for $C_{30}H_{48}N_4Br_3O_3$: C, 47.88; N, 7.45; H, 6.42. Found: C, 47.59; N, 7.39; H, 6.65.

1-[1,3-Bis(butyldimethylammonium)-2-propoxy]carbonylmethyl-3-[(hydroxyimino)methyl]pyridinium tribromide (17(b))

Coupling reaction. The bromoester 80(b) (1.94 g, 3.6 mmol) and 3-pyridine aldoxime (0.04 g, 3.3 mmol) in acetonitrile (15 mL) were stirred at 40°C in a homemade pressure bottle under argon for 3 days. The thick oily residue formed was washed several times with fresh warm acetonitrile and dried under vacuum to give an amorphous solid. This solid was recrystallized in methanol/ethyl acetate to give product 17(b) (0.61 g, 40% yield).

Solubility: MeOH (soluble), CH_3CN (sparingly soluble).

^1H NMR (90 MHz, DMSO-d_6) δ 9.5 (s, 1H), 9.3 (ds, 1H), 9.0 (m, 1H), 8.4 (s, CH=N, 1H), 8.3-8.2 (m, 1H), 5.93 (s, N^+CH_2), 4.2-3.2 (m, 15H), 2.0 (split t, CH_2, 4H), 1.50 (split t, CH_2, 4H), 0.90 (t, CH_3, 6H), Proton exchanged at δ5.93; MS (FAB) m/e 503 ($M^{2+}+{}^{82}Br^-$), 501 ($M^{2+}+{}^{80}Br^-$), 365 (15, 247 (20), 155 (100), 102 (40).

Anal. Calcd. for $C_{23}H_{42}N_4O_3Br_3\ {}_{1/2}H_2O$: C, 40.60; H, 6.52; N, 8.24. Found: C, 40.36; H, 6.59; N, 8.07.

Debenzylation of bezyl ether (attempted). Compound 17(a) (0.5 g, 0.66 mmol) was dissolved in 10 mL of ethanol/water (50:40) and hydrogenated at 30 PSI with 5% Pd/C (375 mg) for 18h. The catalyst was removed by filtration and the filtrate was evaporated to give a green-brown oil. The oil was triturated to give a crude solid (0.36 g). The solid was dissolved in acetonitrile and the insoluble portion was removed by filtration. The solid isolated on evaporation of the filtrate showed ^1H NMR spectrum lacking the benzyl group and the amino alcohol moiety.

This method was repeated in dry methanol varying the catalyst concentration. In all cases, cleavage of the ester group occurred.

1-[1,3-Bis(dodecyldimethylammonium)-2-propoxy]carbonylmethyl-3-[(O-benzyloximinomethyl)]pyridinium tribromide (16(a))

Bromoacylester 89(c) (1.00 g, 1.3 mmol) and 3-pyridine aldoxime (0.40 g, 1.7 mmol) in acetonitrile (15 mL) were stirred in a home-made pressure bottle at 40°C under argon. The reaction was stopped after 3 days, the precipitate was filtered, washed with fresh acetonitrile and dried under vacuum. The solid was recrystallized from chloroform/acetone to give white flaky crystals of 16(a) (0.4 g, 35%).

Solubility: chloroform (soluble), CH_3CN (soluble)

^1H NMR (90 MHz, DMSO-d_6) δ 10.1 (s, 1H), 9.9 (split s, 1H), 8.9 (split s, 1H), 8.55 (s, CH=N, 1H), 8.4-8.0 (m, 1H), 7.5 (s, Ph, 5H), 6.3 (s, N^+ CH_2, 2H), 5.4 (s, CH_2Ph, 2H), 4.5-3.0 (m, 15H), 1.7-1.0 (m, 22H), 0.95 (t, 6H). MS (FAB m/e 563 (40), 270 (45), 214 (50), 93 (60), 84 (100).

Anal. Calcd. for $C_{46}H_{81}N_4O_3Br_3$ H_2O: C, 55.79, H, 8.40; N, 5.62; Br, 24.07. Found: C, 55.79; H, 8.39; N, 5.61; Br, 23.64.

1-[1,3-Bis(dodecyl dimethylammonium)-2-propoxy]carbonylmethyl -3-[(hydroxyiminomethyl)]pyridinium tribromide (16(b))

A solution of bromoacylester 80(c) (0.86 g, 1.12 mmol) and 3-pyridinealdoxime (0.126 g, 1.12 mmol) in dry acetonitrile (10 mL) were stirred at 40°C under argon home-made pressure bottle. The resulting white precipitate (0.51 g) was filtered after 4 days of reaction, washed with fresh acetonitrile and dried. The solid was recrystallized from methanol/ethyl acetate to give white flaky crystals of (0.41 g, 41% yield).

^1H NMR (300 MHz, DMSO-d_6) δ 12.3 (s, N-OH, 1H), 9.4 (s, 1H), 9.15 (s.s, 1H), 8.85 (d, 2H), 8.4 (s, CH=N, 1H), 8.2-8.1 (m, 1H), 5.86 (s, N^+-CH_2, 2H), 4.60-4.50 (m, 4H), 3.7-3.2 (m, 11H), 1.7 (m, 4H), 1.3 (m, 18H), 0.8 (t, 6H). MS (FAB) m/e 435 (200, 418 (30), 332 (35), 212 M^+/3), 84 (100).

Anal. Calcd. for $C_{38}H_{75}N_4O_3Br_3$: C, 52.12; H, 8.63; N, 6.40; Br, 27.37. Found: C, 52.40; H, 8.59; N, 6.15; Br, 27.44.

1[1,3-Bis(dodecyldimethylammonium)-2-propoxy]carbonylmethyl -4-[(hydroxyimino methyl)]pyridinium tribromide (19(b))

A solution of bromoacylester (1.10 g, 1.44 mmol) and 4-pyridine aldoxime (0.20 g, 1.44 mmol) in acetonitrile (10 mL) was stirred in a homemade pressure bottle at 40°C, under argon. The reaction was stopped after 3 days and the precipitated oil was isolated. The solids resulting on addition of ethyl ether were filtered. This crude product was recrystallized from either acetonitrile or methanol/ethyl ether to give 19(b) (32% yield).

Solubility: CH_3CN (sparingly), MeOH (soluble).

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.3 (br, N-OH), 9.45 (ds, 2H), 8.45 (s, CH=N, 1H), 8.3 (ds, 2H), 5.95 (s, N^+-CH_2, 2H), 4.25-3.20 (m, 12H), 1.9 (t, 2H), 1.6-1.4 (m, 20H), 0.95 (t, 6H).

Anal. Calcd. for $C_{38}H_{75}N_4O_3Br_3.H_2O$: C, 52.07; H, 8.68; N, 6.27. Found: C, 51.25; H, 8.37; N, 6.25.

1-[(β-Dodecyldiethylammonium)ethoxy]carbonylmethyl -3-(hydroxyiminomethyl)pyridinium dibromide (18(b))

A solution of bromoester 81 (0.86 g, 1.7 mmol) and 3-pyridinealdoxime (0.19 g, 1.7 mmol) in dry acetonitrile (12 mL) was stirred in homemade pressure bottle at 40°C, under argon. The reaction was continued for 4 days, after which a small amount of ethyl ether was added. The solidified material was separated from the supernatant and dried. The solid product was recrystallized from acetonitrile/ethyl acetate to give 18(b) (0.51 g, 51% yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ9.4 (s, 1H), 9.15 (ds, 1H), 8.8 (ds, 1H), 8.4 (s, CH=N, 1H), 8.2-8.1 (m, 1H), 5.9 (s, N^+-CH_2, 2H), 4.6-4.4 (m, 4H), 3.9-3.3 (m, 6H), 1.8 (t, 2H), 1.6-1.2 (m, 26H), 0.98 (t, 3H).

Anal. Calcd. for $C_{26}H_{47}N_3O_3Br_2$: C, 51.23; H, 7.77; N, 6.89; Br, 26.22. Found: C, 50.89; H, 7.92; N, 6.91; Br, 26.28.

1-[(β-Diethylamino)ethoxy]carbonylmethyl-3-(O-benzyloxyiminomethyl)pyridinium bromide (75) (attempted)

To pyridinium acetic acid 72 (1.0g, 2.85 mmol) suspended in dry methylene chloride (10 mL)/few drops DMF was added oxalyl chloride (0.6 mL, 6.7 mmol) in methylene chloride (5 mL) over 10-15 min at room temperature and stirred for another 30 min. The resulting clear solution was evaporated under reduced pressure to give the acyl chloride and dissolved in fresh methylene chloride (7 mL). To this solution was added diethylamino ethanol (0.34 g, 2.9 mol) in methylene chloride (5 mL) over 25 min and stirred at 40°C for 18 h. The reaction mixture was evaporated to dryness and suspended in ethyl ether. The precipitate that resulted was filtered and dried under vacuum to give a brown solid. ^{1}H NMR of this solid showed unreacted diethylamino ethanol and no coupled product.

Phosphate Diesters and Triesters

Octyl bis(4-nitrophenyl) phosphate (23)

A solution of 1-octanol (4.42 g, 0.034 mol) and dry 2,6-lutidine (3.64 g, 0.034 mol) in dry ethyl ether ("sure seal", 25 mL) was added dropwise over a period of 30 min to a well stirred solution of 4-nitrophenyl phosphodichloride (8.7 g, 0.041 mol) in dry ethyl ether (25 mL) at 0°C, under argon atmosphere. The reaction mixture was stirred overnight at room temperature to allow complete precipitation of lutidine hydrochloride, and the precipitate was removed by filtration. The filtrate was evaporated in a rotory evaporator equipped with a drying tube. The resulting oil (8.85 g) was immediately used in the next step. ^{1}H NMR of the oil perfectly agreed with the structure of octyl 4-nitrophenyl chlorophosphate.

To the above oil (7.58 g, 0.022 mol) in dry ethyl ether (40 mL) was added equimolar sodium nitrophenolate (3.67 g, 0.022 mol) in portions over 1h at 5°C. The mixture was then stirred overnight under argon. The precipitated sodium chloride was filtered off and the solvent was evaporated to give an oil (8.60 g, 73 % yield). The product was handled with great caution.[28]

TLC (solvent system 3): R_f 0.20; ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.4-7.5 (m, 8H), 4.0 (q, 2H), 1.30-0.9 (m, $(CH_2)_6$), 12H), 0.8 (t, CH_3, 3H).

Methyl octyl 4-nitrophenyl phosphate (24)

To methyl dichlorophosphate (5.05 g, 0.034 mol) in pentane (20 mL, dried over molecular sieves) was added 1-octanol (5.49 g, 0.034 mol) and lutidine (3.64 g, 0.034 mol) in pentane (25 mL) over 0.30 min at 0°C, under argon. The white precipitate was filtered in the glove-box and the filtrate was evaporated carefully to give an oil. The product was stored in the glove box.

To this crude oil (4.6 g, 0.019 mol) in dry ethyl ether (25 mL) was added 4-nitrophenolate sodium salt (3.05 g, 0.019 mol) in portions over 1h in a dry box. After the final addition the reaction was stirred for another 2h and the precipitated sodium chloride was removed by a series of filtrations in the glove-box, and washed with ethyl ether. The filtrate was washed with water and dried ($NaSO_4$), and the solvent was evaporated and the resulting oil (23) was stored at -5°C for future use (80 % yield). The product was handled with great caution.[28]

TLC (ethyl ether): R_f 0.78; λ_{max}, pH 9.4: 274 nm. ^1H NMR (90 MHz, $CDCl_3$) δ 8.1 (J_{AB}=8.0 Hz, 2H), 7.30 (J_{AB}=8.0 Hz, 2H), 3.8 (q, 2H), 3.5 (d, CH_3, 3H), 1.40-0.90 (m, $(CH_2)_6$, 0.85 (t, CH_3, 3H).

Octyl 4-nitrophenyl hydrogen phosphate (27)

To the crude oil of octyl 4-nitrophenyl chlorophosphate (1.0 g, 2.86 mmol) obtained during synthesis of 23, was added 2,6-lutidine (25 mL)/water (15 mL) dropwise at 5°C and stirred for 3 to 4h at room temperature. The product was extracted with chloroform (2 x 25 mL), washed with water (2 x 10 mL) and dried (Na_2SO_4). The solvent was stripped off and the resulting oil was washed many times with petroleum either to give the pure lutidine salt of octyl 4-nitrophenyl phosphate (0.77 g, 60% yield).

^1H NMR (90 MHz, $CDCl_3$): δ 8.25 (J_{AB}=8 Hz, 2H), 7.9 (J_{AB}=8 Hz, 2H), 7.6-7.4 (m, 3H), 4.15 (q, 2H), 2.8 (s, $2xCH_3$, 6H), 1.8-1.8 (m, 12H), 0.85 (t, CH_3, 3H).

The lutidine salt was converted to its free acid by stirring in Amberlyst ion-exchange resin (H^+ form) in methanol for 30 min. The resulting solid on evaporation of solvent was recrystallized from methanol/acetonitrile to give white solids of 27.

^1H NMR (300 MHz, CD_3CN) δ 8.06 (J_{AB}=7.5 Hz, 2H), 7.30 (J_{AB}=7.5 Hz, 2H), 3.8 (q, J=10 Hz, $POCH_2$, 2H), 1.35-0.90 (m, $(CH_2)_6$, 12H), 0.8 (t, CH_3, 3H) λ_{max}, pH 9.4: 294 nm. MS (EI) m/e 331 (M^+), 250 (43), 130 (100).

Anal. Calcd. for $C_{14}H_{22}O_6$ NP.¼ H_2O: C, 50.01; H, 6.61; N, 4.17. Found: C, 49.68; H, 6.37; N, 4.60.

Dodecyl 4-nitrophenyl hydrogen phosphate(26)

A solution of 1-dodecanol (2.43 g, 0.0132 mol) and 2,6-lutidine (1.29 g, 0.012 mol) in dry ethyl ether (15 mL) was added dropwise over a period of 30 min to a stirred ice cold solution of 4-nitrophenyl dichlorophosphate (3.0 g, 0.012 mol) in ether (12 mL) under positive argon atmosphere. The resulting white suspension was stirred overnight at room temperature. The precipitate of lutidine salt was filtered, washed with fresh solvent, and the solvent was evaporated to give an oil (dodecyl 4-nitrophenyl chlorophosphate, 4.57 g, 0.012 mol).

The oil was added to lutidine (6 mL) dropwise and stirred at room temperature for 30 min to 45 min, water (30 mL) was added and allowed to stand 3h. The cloudy solution was extracted with chloroform (2 x 15 mL), the organic extract was washed with cold water (2 x 15 mL) and dried (Na_2SO_4). The oil resulting on evaporation of solvent was triturated with petroleum ether (40-60°C) and the residue was dried under high vacuum to give the lutidine salt of the product (3.05 g, 55% yield).

A portion of the salt was converted to the free acid by stirring with ion-exchange resign (Amberlyst, H^+ form) in methanol at room temperature for 2h. The product obtained on evaporation of solvent was recrystallized from ethyl acetate to give 26 as crystals. The product was filtered and dried to yield hydrated free acid.

^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (J_{AB}=8 Hz, 2H), 7.42 (J_{AB}=8 Hz, 2H), 7.0 (br, OH), 4.05 (q, J=9 Hz, $POCH_2$, 2H), 1.75 (q, 2H), 1.4-1.1 (m, 20H), 0.95 (t, CH_3, 3H). MS (EI) m/e 388 (M^+1), 291 (10) 138 (100); λ_{max}

nm (294), (274).

Anal. Calcd. for: $C_{18}H_{30}O_6NP$. $0.5H_2O$: C, 54.53; H, 7.88; N, 3.53. Found: C, 53.68; H, 7.91; N, 3.26.

Butyl 4-nitrophenyl hydrogen phosphate (28)

A solution of 1-butanol (0.46 g, 0.025 mmol) and 2,6-lutidine (0.63 g, 5.86 mmol) in dry ethyl ether (10 mL) was added over a period of 30 min to a stirred solution of 4-nitrophenyl dichlorophosphate (1.5 g, 5.86 mmol) in ethyl ether (10 mL) at positive argon pressure, at 0°C. The resulting white suspension was stirred overnight at room temperature. The precipitate was filtered, washed with fresh solvent, and the solvent was evaporated to give crude butyl 4-nitrophenyl chlorophosphate (1.71 g).

To this crude oil was added lutidine (2 mL), the mixture was stirred for 30 min, and water (15 mL) was added. The cloudy solution was allowed to stand for 6h, extracted with dichloromethane (2 x 20 mL) and washed with water (2 x 10 mL). The organic layer was evaporated to give an oil, the residue was allowed to stand in petroleum ether (40-60°C) overnight and dried under vacuum to give the lutidine salt of 28 (1.19 g, 53% yield).

^1H NMR (90 MHz, $CDCl_3$) δ 8.15 (J_{AB}=7.5 Hz, 2H), 7.26 (J_{AB}=7.5 Hz, 2H), 4.2 (q, J=13 Hz, P-O-CH_2, 2H), 2.9 (s, 2CH_3, 6H), 1.6-1.2 (m, 4H), 0.98 (t, CH_3, 3H).

A portion of the salt was treated with ion exchange resin (Amberlyst, H^+ form) as described in the synthesis of 24. The product isolated was recrystallized from acetonitrile/ethyl ether to give 28.

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.20 (J_{AB}=7.5 Hz, 2H), 7.25 (J_{AB}=7.5 Hz, 2H), 4.15 (q, J=9 Hz, P-O-CH_2, 2H), 1.6 (q, CH_2, 2H), 1.5-1.2 (m, 4H), 0.95 (t, CH_3, 3H). MS (EI) m/e 275 (M^+), 220 (65), 91 (100). λ_{max}, pH 9.4: 274, 294 nm.

Anal. Calcd. for $C_{10}H_{14}O_6NP$. H_2O: C, 41.82; H, 5.32; N, 4.93. Found: C, 41.70; H, 4.85; N, 4.95.

Benzyl 4-nitrophenyl hydrogen phosphate (30)

A solution of benzyl alcohol (0.665 g, 6.15 mmol) and dry 2,6-lutidine in dry ethyl ether (10 mL) was added to a stirred solution of 4-nitrophenyl dichlorophosphate (1.04 g, 5.86 mmol) over 30 min and the solution was stirred overnight at room temperature. The resulting suspension was evaporated to give crude benzyl 4-nitrophenyl monochlorophosphate (1.03 g).

This oil was added to water (20 mL)/tetrahydrofuran (10 mL) for 2h and the pH was adjusted to 4-5 with drops of diluted hydrochloric acid. The solution was extracted with dichloromethane (2 x 25 mL) and dried (Na_2SO_4). Solvent was stripped off to give a semi solid which was then purified through a silicagel column (100-200 mesh) by eluting with solvent system-4 and followed by solvent system 5 to give 30 (43% yield).

^{1}H NMR (300 MHz, CD_3OD/CD_3CN) δ 8.05 (J_{AB}=8.1 Hz, 2H), 7.27 (J_{AB}=8.1 Hz, 2H), 7.6 (s, Ph, 5H), 5.0 (d, J=11 Hz, CH_2 Ph, 2H); MS (EI) m/e 308 ((M-1^+)), 229 (20), 139 (30), 91 (100); λ_{max}, pH 9.4: 294 nm.

Anal. Calcd. for $C_{13}H_{12}O_6NP$. H_2O: C, 47.72; H, 4.31; N, 4.28. Found: C, 47.26; H, 3.89; N, 4.21.

Ethyl 4-nitrophenyl phosphate (29)

A solution of absolute ethanol (0.27 g, 5.86 mmol) and 2,6-lutidine (0.63 g, 5.86 mmol) in dry ethyl ether (10 mL) was added to a stirred solution of 4-nitrophenyl dichlorophosphate (1.04 g, 5.86 mmol) over 30 min at 0°C and the solution was stirred overnight. The resulting white precipitate was filtered off and washed with fresh solvent. The solvent was stripped off to give ethyl 4-nitrophenyl chlorophosphate.

This oil was dissolved in tetrahydrofuran (10 mL) and added to cold water (20 mL) with a few drops of diluted hydrochloric acid. The solution mixture was extracted with methylene chloride (2 x 20 mL) and dried (Na_2SO_4). The solvent was stripped off, product residue was recrystallized from ethyl acetate/petroleum ether (40-60°C) to give beige crystals of 29 (13% yield).

^1H NMR (300 MHz, $CDCl_3$) δ 9.5 (br, OH), 8.24 (J_{AB}=7.7 Hz, 2H), 7.36 (J_{AB}=7.7 Hz, 2H), 4.25 (q, J=9 Hz, $POCH_2$, 2H), 1.4 (t, J=9 Hz, CH_3, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.3, 144.8, 125.7, 120.71/120.63, 65.36/65.29, 15.98/15.90; MS (EI) m/e 247 (M^+), 167 (55), 109 (100); λ_{max}, pH 9.4: 292 nm.

Anal. Calcd. for $C_8H_{10}O_6NP$: C, 38.87; H, 4.07; N, 5.67. Found: C, 38.67; H, 4.09; N, 5.60.

Bis(2,4-dinitrophenyl) phosphate (33)

(i) Literature method[73] (attempted). Phosphoryl chloride (1.53 g, 0.01 mol) was added slowly under argon to a well stirred solution of 2,4-dinitrophenyl phosphate (5.52 g, 0.03 mol) in 2,6-lutidine (50 mL) at room temperature. The solution was then warmed to about 50°C for 30 min then allowed to cool for 1h while stirring. The mixture was poured on to water (100 mL) and the dark solids were filtered off and washed with water. Recrystallization from ethanol (charcoal) give 0.2 g of faint beige product. The product showed violet colorization on addition to alkali. The m.p. (200-202°C) failed to agree with the literature value (178-179°C) even after several recrystallizations. The properties of this compound resemble those of pyridinium salts of 2,4-dinitrophenol. The procedure was repeated several times without any success.

(ii) Literature method[74] (attempted). To a suspension of 2,4-dinitrophenol (5.25 g, 0.064 mol) and dry pyridine (18 mL) was added phosphoryl chloride over 30 min, the mixture was stirred at room temperature for 3h and added to deionized water (200 mL), with stirring. The reaction mixture was cooled, precipitate was filtered and washed with water, and the crude product was dried over P_2O_5 under vacuum. The resulting yellow crystals were recrystallized from water. The product showed violet colorization on addition to alkali, indicative of a pyridinium salt of 2,4-dinitrophenol but not the desired product.

(iii) Modified method. To 2,4-dinitrophenol (2.76 g, (recryst. from toluene), 0.03 mol) in 2,6-lutidine (99%, 25 mL) and dry acetonitrile (15 mL) was added to phosphoryl chloride (distilled, 0.5 mL, 1.53 g, 0.01 mol) via a syringe at room temperature, under argon. The solution was warmed to about 45°C for 15 min. The resulting dark solution was stirred at room temperature for 30 min and then allowed to cool in a ice-bath for 1h under stirring. The reaction mixture was poured into ice-water (50g) and water (150 mL) was further added. About 75 mL of water was stripped off in a rotory evaporator at 55°C and the resulting dark precipitate was filtered and washed with water. Crude product was dried under vacuum overnight and recrystallized from either ethanol or acetonitrile to give colorless crystals of 33 (39% yield): m.p. 181-182°C (lit. 184-186°C[73]).

^{1}H NMR (90 MHz, $CDCl_3$) δ 8.9-7.65 (m, 9H), 2.5 (s, $2xCH_3$, 6H); λ_{max}, pH 9.3: 265 nm.

2,4-Dinitrophenyl 4-nitrophenyl hydrogen phosphate (31)

A solution of 2,4-dinitrophenol (1.10 g, 6.00 mmol) and 2,6-lutidine (0.63 g, 5.86 mmol) in ethyl ether (15 mL) was added over 30 min to a stirred solution of 4-nitrophenyl dichlorophosphate in dry benzene at room temperature, under argon. The reaction mixture was allowed to stand overnight, the precipitate was filtered off and washed with fresh solvent. The filtrate was evaporated under reduced pressure to give an oil (0.94 g).

Lutidine (3 mL) was added dropwise to the oil, stirred for 30 min and water (30 mL) was added. The solution was left overnight at +5°C extracted with chloroform (2 x 20 mL), and washed with water (2 x 10 mL). The solvent was stripped off and the resulting oily residue was suspended in petroleum ether (40-60°C). TLC (solvent system-1) showed three components with one of them as the major product. ^{1}H NMR of the product mixture indicated the presence of the desired product. Further isolation of product was not carried out.

Oxazolidinones and Glycourils

4-Hydroxymethyl-4-methyl-2-oxazolidinone (44)[76]

A solution of urea (7.2 g, 0.19 mol.) and 2-amino-2-methyl-1,3-propanediol (Aldrich, 12.6 g, 0.141 mol.) in dimethylformamide (18.6 g) was refluxed for 12h The reaction mixture was poured on to ethyl ether (400 mL) and stirred for 1h. The resulting white precipitate was filtered and recrystallized from methanol-acetone to give 44 (11.3 g, 60% yield): mp 115-116°C.

^{1}H NMR (90 MHz, D_2O) δ 4.41 and 4.0 (J_{AB}=8.5 Hz, 2H), 3.5 (s, 2H), 1.3 (s, 3H); ^{13}CNMR (74.4 MHz, DMSO-d_6) δ 158.76 (C=O), 72.20, 66.60, 58.40, 22.66.

Anal. Calcd. for $C_5H_9N_1O_2$: C, 45.80; H, 6.92; N, 10.68 Found: C, 45.88; H, 6.93; N, 10.68.

Pyridinium(4-methyl-2-oxazolidinone-4-yl)methyl sulfate (46(a))

To a hot solution of 44 (1 g, 7.6 mmol) in warm acetonitrile (10 mL) was added pyridine sulfoxide (1.23 g, 7.75 mmol) in portions under dry conditions. The reaction was allowed to stir at 50°C for about 1h and then allowed to stand overnight without further stirring. The solvent was evaporated and the resulting oil was washed sequentially with (1) chloroform-acetone 1:2 (15 mL), (2) acetone-acetonitrile 1:2 (2x15 mL), (3) acetone:acetonitrile 1:1 (10 mL) and the residual oil was evaporated to dryness. The residual oil was dissolved in water (10 mL) and evaporated to dryness to give a brown syrupy oil of 46(a) (1.2 g, 69% yield).

^{1}H NMR (90 MHz, D_2O) δ 8.8-7.9 (M, 5H, Pyr), 4.4 and 4.0 (J_{AB}=9 Hz, 2H), 3.9 (S, 2H, $\underline{CH_2}O$), 1.3 (S, CH_3).

Anal.Calcd. for $C_{10}H_{14}N_2SO_6$. H_2O: C, 38.96; H, 5.23; S, 10.40; N, 9.09. Found: C, 39.00; H, 5.14; S, 10.49; N, 9:02.

Sodium(4-methyl-2-oxazolidinone-4-yl)methyl sulfate (46(b))

The pyridinium salt of sulfate 46(a) (2.10 g, 7.26 mmol) was dissolved in water (2 mL) and sodium hydroxide (0.40 g, 10.00 mmol) was added and stirred at room temperature overnight. The solvent was evaporated to dryness and ethanol (5 mL) / water (2 mL) was added. The precipitated inorganic salt was removed and the filtrate was further precipitated by adding acetone on cooling. The filtrate was evaporated to give a white hygroscopic solid and the solid was dried under vacuum to give the mono sodium salt of the sulfate 46(b) (0.7 g, 42% yield).

Anal. Calcd. for $C_5H_8O_6SNNa$. H_2O: C, 23.90; H, 4.01; N, 5.58; S, 12.76; Na, 9.15. Found: C, 24.28; H, 3.93; N, 5.30; S, 12.73; Na, 9.10.

3-Bromo-4-hydroxymethyl-4-methyl-2-oxazolidinone (45)

To a solution of 44 (0.66 g, 5 mmol) and sodium hydroxide (0.28 g, 7 mmol) in 5 mL of water was added bromine (0.88 g, 5.5 mmol) at 0°C with stirring. After 30 minutes the precipitate was collected by filtration, washed with a small amount of cold water and dried (Na_2SO_4) to give a white crystalline precipitate identified by ^1H NMR as the desired product. A small amount of product was dissolved in DMF and analyzed (iodometry) to have 85-90% bromine content per mole product. The bromine content decreased with storage time.

^1H NMR (90 MHz, $CDCl_3$/DMSO-d_6) δ 4.30 (d, H), 3.93 (d, 1H), 3.43 (S, 2H), 1.30 (S, 3H).

Anal. Calcd. for $C_5H_8BrNO_3$: C, 28.59; H, 3.84; N, 6.67; Br, 38.05. Found: C, 29.34; H, 3.85; N, 6.81; Br, 36.46.

(3-Bromo-4-methyl-2-oxazolidinone-4-yl)methyl sulfate (47)

(i) Bromination by bromine/Sodium Hydroxide. To 46(b) (0.71 g, 2.83 mmol) dissolved in water (2 mL) was added sodium hydroxide (0.1132 g, 2.83 mmol) and the mixture was stirred room temperature for 15 min before the solution was brought to -5°C. Bromine (0.5 g, 4.25 mmol) was added to the above solution under stirring and stirred further for 20 min at 0°C. The resulting yellow-brown suspension was filtered through a medium pore sintered glass funnel and the filtrate was freeze-dried to give a faint yellow solid. The solid was examined by iodometric titration to have 86-90% of bromine content per mole. Attempted removal of sodium bromide from the product mixture by t-butanol/acetonitrile solvent wash reduced the bromine content to 40-50%.

(ii) Bromination by NBS. To 46(b) (0.5 g, 2.1 mmol) in water (1.5 mL) was added NBS (0.39 g, 2.2 mmol) at -5°C and the mixture was stirred for 15 min. The suspension was filtered through a medium pore sintered glass funnel and the filtrate was washed with cold carbon tetrachloride (2x15 mL). The aqueous layer was freeze-dried to give a faint yellow solid. The bromine content was determined by iodometric titration to be 70-75%. The ^{1}H NMR of the solid showed the skeleton of the oxazolidinone ring and peak for succinimide (δ 2.9 ppm).

(iii) Bromination by t-butylhypobromite. To a stirred solution of bromine (0.64 g, 0.04 mol.) in water (6 mL) was added silver sulfate (9.6 g, 0.048 mol.) in portion until the dark brown color changed to orange (≈15 min). The aqueous part of the solution was decanted through a glass wool into a flask containing t-butanol (2.66 g, 0.036 mol.) in fluorocarbon II ($CFCl_3$, 15 mL) in a cold water bath and shaken over 15 to 20 min. The organic layer was separated and dried (Na_2SO_4). The solvent was evaporated under low vacuum at air temperature to give a yellow-red oil (t-butylhypobromite, 1.0 g). This oil showed 77% bromine incorporation by iodometric analysis. This oil was immediately carried on to the next step or stored at -5°C.

To a solution of 46(b) (0.6 g, 2.6 mmol) and water (3 mL) at 0-5°C was added t-butylhypobromite (77%, 0.85 g, 3.0 mmol) and the mixture was stirred for 45 min. The reaction mixture was filtered through a sintered glass funnel and the aqueous filtrate was freeze-dried to give faint yellow solid (0.8 g, 97%). The bromine content was determined iodometrically to be 100-104% per mole. The sample was stored in an amber vial in a desiccator. The ^1H NMR was recorded (quickly) in deuterium oxide, and showed a slight change in the AB splitting pattern of the oxazolidinone ring compared to that of the parent free amine. The sample was noticeably decomposed on standing in deuterium oxide.

^1H NMR (60 MHz, D_2O) δ 4.2 and 4.4 (J_{AB}=7 Hz, 2H), 3.9 (s, 2H), 1.3 (s, 3H).

Anal. ($C_5H_8NO_6SBr$) H, N; C: Calcd. 27.89; Found, 27.60.

4-Succinoyloxymethyl-4-methyl-2-oxazolidinone (48)

To 44 (1 g, 7.7 mmol) dissolved in hot acetonitrile (7 mL) was added succinic anhydride (0.88 g, 8.6 mmol) in portions at 50-60°C under argon atmosphere, and pyridine (0.77 g, 7.7 mmol) was added to the above solution and stirred for 1h The reaction mixture was allowed to stir at room temperature for 4 to 5h.

The solvent was evaporated to dryness and water (5 mL) was added, and the solution was evaporated in a warm water-bath to give a syrupy oil. The oil was dried under high vacuum to give 48 (1.3 g, 73% yield).

^{1}H NMR (90 MHz, $CDCl_3$) δ 6.7 (br, NH), 4.5-4.0 (AB + s, 4H), 2.5 (s, $(CH_2)_2$, 4H), 1.4 (s, CH_3, 3H). MS (EI) m/e 231 (M^+), 133 (45), 100 (100).

Anal. Calcd. for $C_9H_{13}O_6N$: C, 46.76; H, 5.68; N, 6.02. Found: C, 47.94; H, 5.94; N, 6.27.

3-Bromo-4-succinoyloxymethyl-4-methyl-2-oxazolidinone Sodium Salt (49)

To a solution of 48 (0.56 g, 2.17 mmol) in water (3 mL) was added sodium hydroxide (0.213 g, 5.22 mmol) and the mixture was stirred for 30 min. To this solution was added bromine (0.35 g, 2.17 mmol) dropwise at 0-5°C until a brown color persisted. The resulting yellow suspension was further stirred for 30 min and the precipitate was filtered. The precipitate was dried to give 49 (0.26 g, 36% yield). This product was analyzed by iodometric titration to have 98 to 100% bromine incorporation per mole of product. The aqueous portion was freeze-dried, and it was found to contain inorganic salts and ring opened products.

^{1}H NMR (60 MHz, DMSO-d_6) δ 4.6-4.1 (AB + s, 4H), 2.5 (s, 4H), 1.4 (s, CH_3, 3H). MS (EI) m/e 253 ($(^{82}M-CH_2CO_2^-)^+$), 251 ($(^{80}M-CH_2CO_2^-)^+$), 173 (11), 100 (100).

4-(β-Diethylamino)ethyloxymethyl-4-methyl-2-oxazolidinone (50)

To a suspension of sodium hydride (1.89 g, 60%, 0.047 mmol) in dry DMF (5 mL) was added 44 (3 g, 0.023 mmol) over 30 min at room temperature and the resulting suspension was warmed for 1.5h at 50-60°C. To this was added chloroethyldiethylamine (0.023 mmol) in DMF (5 mL) over 25 min at room temperature, the reaction mixture was warmed at

60°C for 2h and allowed to stand overnight. The resulting mixture was poured into ice/water and acidified carefully with 2M HCl to pH 4-5 and extracted with chloroform (2x50 mL). The aqueous layer was brought to pH 9-10 with 50% sodium hydroxide solution and extracted with chloroform (2x25 mL). The combined chloroform layer was washed with saturated sodium bicarbonate (2x25 mL) and dried (Na_2SO_4). The solvent was evaporated to give an oil. The oil was suspended in hexane to remove the mineral oil and remaining oil was eluted through a silica gel column (100-200 Mesh) with solvent system 1 and then with solvent system 2 to give two major components at R_f 0.54 and 0.28 (TLC; solvent system-1) in 8:2 ratio. The high R_f component was found to be mono O-alkylated product 50 (1.95 g, 33% yield); mp 74-75°C. The low R_f component (oil) was found to be dialkylated product (9% yield).

High R_f component (50): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.5-7.4 (br, NH), 4.5 and 4.0 (J_{AB}=9 Hz, 2H), 3.4-2.85 (m, 10H), 1.5-1.3 (m, 9H); ^{13}C NMR (74.5 MHz, DMSO-d_6) δ 158.23 (C=O), 70.17 (3rd C), 63.14 (ring CH_2), 62.79 (CH_2O), 51.28 (OCH_2CH_2), 47.17 (CH_2), 37.97 (CH_2), 20.14 (CH_3), 10.50 (CH_2CH_3). IR (neat) 3200 (b, N-H), 1740 (s, C=O), 750 (N-H out of plane) cm^{-1}. MS (EI) 230 (M^+), 158 (10), 100 (10), 86 (100).

Anal. Calcd. for $C_{11}H_{23}O_3N_2$: C, 57.24; H, 9.83; N, 12.14. Found: C, 57.23; H, 9.83; N, 12.09.

Low R_f component; ^{1}H NMR (90 MHz, $CDCl_3$) δ 4.5 and 4.0 (J_{AB}=9 Hz, 2H), 3.4-2.85 (m, 18H), 1.5-1.3 (m, 15H); MS (EI) m/e 329 (M^+), 300 (20), 229(18), 86(100); IR (neat) 1740 (C=O) CM^{-1}.

Anal. Calcd. for $C_{17}H_{35}O_3N$: C, 61.97; H, 10.71; N, 12.75. Found: C, 70.83; H, 10.72; N, 12.27.

4-(β-Methyldiethylammonium)ethyloxymethyl-4-methyl-2-oxazolidinone iodide (51)

Compound 50 (0.25 g, 1.1 mmol), methyl iodide (0.213 g) and sodium carbonate (few crystals) were stirred in dry chloroform (5 mL) overnight. The solvent was evaporated, the residue was dissolved in methanol and insoluble substances were filtered off. The filtrate was evaporated and the resulting white solid was crystallized from methanol/ethyl acetate to give 51 (76% yield).

^{1}H MNR (90 MHz, D_2O) δ 4.5 and 4.0 (J_{AB}=8 Hz, 2H), 3.6-3.2 (m, $(CH_2)_n$, 10H), 3.1 (s, N^+CH_3, 3H), 2.5-1.4 (m, 6H). MS (FAB) m/e 245 (M^+, 100), 158 (35).

Anal. Calcd. for $C_{12}H_{25}O_3N_2IO$. H_2O: C, 37.78; H, 6.83; N, 8.34. Found: C, 37.82; H, 6.63; N, 7.15.

4-(β-Dodecyldiethylammonium)ethyloxymethyl-4-methyl-2-oxazolidinone bromide (52)

The solution of compound 50 (0.1 g, 0.43 mmol) and bromododecane (0.3 g, 1.4 mmol) in CH_3CN (5 mL) was heated at refluxed for 24h. The volume of the solvent was reduced to one quarter original and ethyl ether was added. White crystals of 52 were collected (0.44 g). ^{1}H NMR (90 MHz, CD_3CN) δ 5.1-5.05 (br, NH), 4.3-3.85 (J_{AB}=8 Hz, 2H), 3.8-3.15 (m, 12H), 1.8-1.11 (m, 29H), 0.8 (t, 3H). MS (FAB) m/e 399 (M^+, 100), 367 (8), 155 (10).

N-Benzoxyformoyl-4-hydroxymethyl-4-methyl-2-oxazolidinone (86)

To a solution of 50 (2 g, 15.3 mmol) and sodium carbonate (0.8 g, 15.3 mmol) in water (10 mL) was added chlorobenzylformate (2.6 g, 15.3 mmol) at 5°C and the solution was stirred for 45 min. The product was extracted with methylene chloride (2x25 mL) and the solvent was evaporated to give an oil. The oil was kept under high vacuum at 60°C to remove the residual benzyl alcohol. The resulting oil was chromatographed through silica gel (100-200 Mesh) using solvent system 1 to give a colorless oil of 84 (70% yield).

^{1}H NMR (60 MHz, $CDCl_3$) δ 7.4 (s, Ph, 5H), 4.4-3.9 (AB and s, 4H), 1.30 (S, 3H). MS (EI) m/e 266 (M+1), 191 (30), 100 (80), 91 (100).

4-Malonyloxymethyl-4-methyl-2-oxazolidinone (86) (attempted)

To compound 44 (1.0 g, 7.6 mmol) in CH_3CN (2 mL) was added malonyl chloride (0.56 g, 7.6 mmol) and stirred at 50°C for 4h The reaction mixture was poured onto ice (5 g) and the aqueous layer was washed with chloroform (2x15 mL). The aqueous layer was evaporated to dryness to give an oil. The ^{1}H NMR of the crude oil showed the presence of product (86), but it was not pure enough to isolate the product easily. Attempts to crystallize the product from the crude oil were not successful.

The above reaction was atttempted under various conditions in the presence of pyridine, triethylamine and pyridine and each time no desired product was obtained.

4-Methyl-2-oxazolidinone-4-methyloxyacetic acid (87) (attempted)

(i) Use of sodium hydride. To a suspension of sodium hydride (60%, 3.12 g, 0.078 mol) in dry DMF (15 mL) was added 44 (5.0 g, 0.038 mol) in DMF (20 mL) over 30 min under positive argon atmosphere, at room temperature. The reaction mixture was warmed at 70°C for 2h, and allowed to stand overnight.

The reaction mixture diluted with methanol and insoluble components were filtered. The filtrate was evaporated to dryness, the solids were dissolved in water and acidified to pH 4 with concentrated hydrochloric acid. The solution was washed with ethyl ether and the aqueous layer was evaporated to dryness to give dark solids. ^{1}H NMR of the solid showed the presence of mixture of products including the desired one. The crude solid was suspended in acetone and the insoluble portions were filtered. The filtrate was evaporated to give a thick oil and the ^{1}H NMR of this oil showed a pattern for two products and unreacted starting materials. Further isolation and purification of the desired product from the pool of products was not attempted.

(ii) Use of n-Butyllithium (n-BuLi). The above reaction carried out with n-butyllithium in HMPA/THF produced ring opened products (^{1}H NMR).

(iii) Reaction with neat iodoacetic acid. The reaction (i) was carried out with iodoacetic acid in the absence of a base, but gave no appreciable reaction.

3a,7a-Dimethyltetrahydroimidazo[4,5-d]imidazole-2,5-dione[50]
(54(a), dimethylglycouril)

Urea (35 g, 0.58 mmol) and methyl diketone (20 g) was stirred with water at room temperature. for 3 to 4 days. The resulting white precipitate was filtered and washed with excess water. The crude solid was suspended from hot dimethylsulfoxide, filtered and washed with excess methanol to give 54 (30 g, 61%).

^{1}H NMR (90 MHz, DMSO-d_6) δ 7.0 (br, NH), 1.3 (s, CH_3); ^{1}H NMR (90 MHz, TFA) δ 7.0 (br, NH), 1.3 (s, CH_3); MS (EI) m/e 171 ($(M+1)^+$), 129 (27), 111 (65), 86 (100).

1,3,6,8-Tetrabromo-3a,7a-dimethylhydroimidazo[4,5-d]imidazole-2,5-dione (54(b))

To 54(a) (0.85 g, 5 mmol) and sodium hydroxide (1.12 g, 28 mmol) in water (5 mL) was added bromine (3.5 g, 22 mmol) at 5°C and the mixture was stirred for 20 min. The resulting faint yellow precipitate was filtered and washed with fresh water, and the product was dried under vacuum to give 54(b) (1.4 g, 58%). The product was found to be only soluble in warm methanol with decomposition, trifluoroacetic acid, warm water (sparingly soluble). The product reacted rapidly on addition dimethylsulfoxide-d_6. The ^{1}H NMR spectrum was obtained a few minutes after preparation of sample with deuterated methanol confirmed the structure. m.p. 187-193°C (decomp.). Iodometric titration showed 4 mole bromine per mole of compound 54(b).

^{1}H NMR (60 MHz, CD_3OD) δ 1.5 (s, 2 x CH_3).

Monosubstitution of dimethylglycouril (attempted)

(i) Use of formaldehyde. Formaldehyde (30 mL, 37% HCHO) and glycouril (5 g, 29.4 mmol) in water (130 mL) were stirred overnight. The resulting solution was filtered and the filtrate was evaporated to dryness give an amorphous solid. The ^{1}H NMR spectrum of the solid showed that polyhydroxymethylation had occurred. The product mixture decomposed on standing at room temperature for 3 to 4 days.

^{1}H NMR (90 MHz, D_2O) δ 4.4 (s, ≈8H), 1.4 (s, 4H), 1.3 (s, 2H).

Method (i) was carried out with equimolar formaldehyde at various conditions. The reaction mixture showed polyhydroxylated and unreacted 54(a) was obtained (^{1}H NMR).

(ii) Use of bromoacetate. Dimethyl glycouril (5 g, 29.4 mol) was added instantly to sodium hydride (60%, 0.30 g, 7.35 mmol) in DMF (20 mL) and stirred for 1h at 50°C. The solution was warmed for another 2h at 60-70°C and brought to room temperature. Bromoacetate ester was added to above suspension over 20 min and heated to 60-70°C for 3h. The reaction was followed by TLC (methanol/ethyl acetate/ammonia 15:80:5).

The reaction mixture was filtered and the filtrate was evaporated to give a crude semi-solid, which was precipitated with addition of acetone. The ^{1}H NMR of the solid showed only a singlet at δ 3.5, but lacked CH_3 and OCH_2CH_3 signals of the product. The product thus isolated decomposed on standing at room temperature.

Repeated attempts using potassium carbonate, LDA and n-BuLi under various conditions failed to yield the desired product.

(iii) Use of succinic anhydride. Succinic anhydride (1.47 g, 14.7 mmol) and 54(a) (2.5 g, 14.7 mmol) in pyridine (10 mL) were stirred under dry conditions at 40-50°C over 2 days. The reaction was stopped and the insoluble portion was filtered. The precipitate and filtrate were dried under vacuum. The spectral data (^{1}H NMR) were not indicative of the desired product. The product obtained from evaporation of pyridine showed the presence of succinic acid pyridinium salt.

(iv) Use of N,N-dimethyleneammonium iodide (Eschenmoser's salt). Eschenmoser's salt (1.11 g, 6 mmol) was added to 54(a) (1 g, 6 mmol) in methanol (10 mL) and followed by a few drops of acetic acid. The reaction mixture was stirred at room temperature overnight and allowed to continue for another day at 50°C. Unreacted starting material was filtered out and the filtrate was evaporated to give a faint yellow solid. ^1H NMR of the solid showed singlets at δ 4.4 and δ 2.5, not indicative of the coupled product. Mass spectral data, m/e 323 (M^+), 219 (100) did not correspond to the expected product.

The above procedure was repeated with acetonitrile as the solvent and in the absence of acetic acid. The product(s) isolated showed spectral data that were unreasonable for the desired product.

Physical-Chemical Studies

Excessively Charged Nucleophilic Reactivators

Preparation of buffer solution

Sodium acetate buffer (0.5 M), pH 5.36 was prepared by dissolving 6.81 g sodium acetate (NaAc. $2H_2O$) in 100 mL of deionized water containing 0.2 mL of concentrated sulfuric acid.

Borate buffer (0.2 M), pH 8.3 was prepared by dissolving 3.025 g of boric acid and 0.1905 g of sodium borate in 100 mL of deionized water.

Bicarbonate/carbonate buffer (0.2 M); pH 9.0 to 10.8 was prepared by mixing sodium carbonate (0.2 M) and sodium bicarbonate (0.2 M) together.

The pK_a determination of hydroxamic acid and oxime ECNR compounds

The pK_a of ECNR (14 to 19) were determined spectrophotometrically at fixed reagent and buffer concentrations in the absence of co-surfactants. Carbonate-bicarbonate and phosphate buffers ranging from pH 5.0 to 12.0 were prepared at 0.03M and 0.05M concentrations.

General method: A constant volume (100-200 μL) of aliquot from a fixed stock solution of ECNR was added to 2.5 mL of buffer solution in a UV cell and equilibrated at 35°C ± 1°C for about 30 min. The absorption spectra of equilibrated solutions were determined from 190 to 600 nm at different pHs. The pH of the solution was determined before and after photometric measurements in order to ensure a constant pH.

The absorbance maxima for the anionic species were plotted against pH of the buffer solution. Sigmoidal curves were obtained and pk_a values were calculated based on three determinations around the point of half-neutralization using the equation[15]:

$$pk_a = pH_{obs} + \log \frac{[A_{max} - A_{obs}]}{[A_{obsd} - A_{min}]}$$

Where A_{max}, A_{min} and A_{obsd} are respectively the absorbance values of the completely ionized, undissociated and the partially dissociated (absorbance at pH measured) of a nucleophile under the concentration investigated.

Critical micelle concentration determination of ECNR.

A predetermined amount of concentrated stock solution ($\approx 10^{-1}$ M) of a ECNR compound was micropipeted into a UV cell containing 2.5 mL of buffer (fixed strength). The solution was equilibrated for 20 min and using a spectrofluorometer the solution was excited at a wavelength, and the emission intensity was recorded at its $_{max}$. The emission intensity was plotted against the concentration of ECNR and the inflection point in the curve determined as the critical micelle concentration.

Kinetic studies of organophosphate triesters by ECNR

Kinetic studies of organophosphate triesters by ECNR

The stock solution of oximes (16 to 19) and hydroxamic acids (14, 15) were made by dissolving accurately weighed (about 0.05 g) amount of pure samples in deionized water (2 or 4 mL) to give a concentration around 3 x 10^{-2}M. The oxime (21, 22) were dissolved in 50% v/v water:ethanol. The 1% v/v solutions of Paraoxon and octyl methyl 4-nitrophenyl phosphate were made from pure samples by dissolving in dioxane and stored in sealed ampule's at +5°C.

Hydrolysis of Paraoxon and octyl methyl 4-nitrophenyl phosphate produces p-nitrophenolate ion (λ_{max} = 400 nm). Therefore, absorbance at 400 nm was followed spectrophotometrically, using a Hewlett Packard 8451 Diode Array spectrometer equipped with a microprocessor. All reactions were carried out in buffer systems of ionic strength 0.12 or 0.25 (0.03M and 0.05M). The concentration of ECNR was varied from 1 x 10^{-4} M to 10^{-3} M, maintaining a constant phosphate triester concentration (5.0 to 6.5 x 10^{-5} M) in order to provide pseudo first order reaction conditions.

General method 1. A predetermined amount of concentrated ECNR solution was micropipeted to 2.4 mL buffer and equilibrated for 1h in the thermostatted UV cell holder at the temperature investigated. The reaction was started by adding 2 to 5 μL of 1% phosphate ester solution. The absorbance of product was measured at every 0.25 or 0.5 or 1.0 or 5.0 or 10.0 min intervals with the use of a Basic kinetic program controlled by a microprocessor, run on the spectrometer. The reaction was followed for a minimum of 3 half lives (or 4h). The rate constants were calculated from half lives obtained from semi-logarithmic correlation of A_∞-A_t against time (A_t is the absorbance at time t and A_∞ the end absorbance of the reaction) from the data obtained over 60 min of reaction, using a Texas Instrument T1-5511I programmable calculator. The A_∞ was measured, in general, after 98% completion of the reaction or in some cases, calculated from the calibration plot of absorbance (A) against concentration of 4-nitrophenol.

All experiments were run in duplicate and tabulated data represent the average of these experiments. A correlation coefficient of 0.998 ± 0.001 was obtained at each run. The standard deviation between each trial was less than 5% The buffer hydrolytic rates for each of the phosphate esters were measured at each temperature and pH investigated, when not negligible these values were subtracted from the rate of nucleophilic (ECNR) hydrolysis. The pH of the reaction medium was checked before and after reaction to make sure that the pH remained constant.

Owing to the extreme toxicity of both Paraoxon and octyl methyl 4-nitrophenyl phosphate the reactions were carried out in screw capped UV cells sealed with parafilm. The addition of phosphate ester to the reaction cell was made under high draft hood and all glass apparatus was handled with thick rubber gloves. All syringes, glassware and UV cells that used were decontaminated with 2% aqueous NaOH.[28,35]

Effect of temperature. The method described above for the pseudo first order process was carried out at different temperatures such as 25°C and 35°C. In cases of turbidity, a very small amount (2 to 3 μL) of 0.2M CTAB was used to bring clarity to the solution.

Effect of cetyltrimethylammonium bromide (CTAB). A stock solution (0.2 M) of CTAB was made by dissolving 18.223 g of pure sample (Aldrich) in 250 mL of deionized water. The effect of CTAB on the reaction rate was investigated using general method 1 below and above the CMC, by adding a predetermined amount of CTAB solution to the reaction medium prior to the addition of phosphate esters. The rate constants were calculated as described in Method 1.

The pH rate profile. The kinetic run was carried out using method 1 at various buffer pHs with a fixed buffer concentrations. A series of buffer solutions at range of pH 7.5 to 11.2 were made from concentrated buffers with appropriate dilutions. The observed rate constants measured were corrected for buffer hydrolysis if not negligible and releated to the active nucleophile (anionic species) concentration, at the pH investigated.

Determination of order kinetic process

The procedure was essentially the one used in method 1. The relative concentration of [ECNR] ≈5 or 3 x [phosphorus ester]. Reaction was allowed until a clear cut non-linearity was seen in the first order process. With rapid reactions, following complete destruction of phosphate ester an additional quantity of phosphate ester was added to the reaction mixture and the reaction rate was determined. The semilogarithmic plots obtained on plotting $A_\infty - A_t$ against time, in all cases, indicated non-linear rate profiles. Second order rate constants were obtained from the slope of the plot of 1/[phosphat substrate] against time.

Kinetic studies of the hydrolysis of phosphate diester and phosphonate monoanions (aged AchE models) by ECNR compounds.

Stock solutions (10^{-2} M) of alkyl (or aryl) 4-nitrophenyl phosphates, phenyl 4-nitrophenyl phosphonate and Bis-2,4-dinitrophenyl phosphate were made by dissolving accurately weighed (~0.03 g) pure sample in methanol (1.0 to 2.0 mL). The stock solutions were stored at +5°C in sealed vials in order to minimize self decomposition.

All reactions were carried out essentially using methods 1 or otherwise stated at constant buffer concentrations, and at constant pH in the range of 8.0-10.0. The concentrations of phosphate diester used (6×10^{-5} to 1×10^{-4} M) were slightly higher than those used for phosphate triesters, but maintained pseudo first order reaction conditions. All kinetic runs except bis-2,4-dinitrophenyl phosphate hydrolysis were monitored at 400 nm. Slow reactions and buffer only hydrolysis of diesters were monitored over 7 to 12 days, in some cases over a month, in a water-bath set at the temperature investigated.

The end point absorbances (A_∞) of slow reactions (half life > 10 days) were estimated from the calibration curves by plotting absorbance (A) against concentration of 4-nitrophenol at conditions investigated. First order rate constants were calculated mostly from the half lives obtained from semilogarithmic plot of A_∞-A_t against time or by the "initial rate method" from the slopes of the linear plots of optical density against time by converting to concentration units (ϵl= 17,925 at pH 9.3) and dividing by initial phosphate ester concentration. Kinetic data tabulated represents the average of two of these experiments. The correlation coefficient for each determination was 0.996 ± 0.003. The standard deviation between each trial was less than 5%.

A minimum amount (2 to 3 μL) of CTAB (0.2M) was added to reaction mixture, in some cases, in order to clear turbidity of the solution without altering the reactivity of ECNR.

Rate measurement of bis-2,4-dinitrophenyl phosphate (33) (method 2). A slightly different procedure than the one used for other phosphate diesters was applied to investigate the hydrolysis of bis-2,4-dinitrophenol phosphate. Hydrolysis of bis-2,4-dinitrophenyl phosphate to inorganic salt is a two stage (bi-phasic) reaction for which rate constant, K, of the first stage is required. Under the conditions investigated Phase I was found to be more rapid than Phase II. Therefore, the end point of Phase I, conversion of diester anion to monoester dianion, was determined using a calibration curve plotting absorbance against concentration of 2,4-dinitrophenol. In an alternative approach, 10 μL (0.1086 units) of E. coli alkaline phosphatase (Sigma) in 0.01 M $(NH_4)_2SO_4$ was added to the aged reaction mixture so that any 2,4-dinitrophenyl phosphate was rapidly hydrolyzed.[74] The A_∞ for the Phase I reaction was taken as half of the absorbance (at 350 nm) measured at the end of the reaction. A_∞ calculated by both the methods were in close agreement. A semilograph plot of A_t against time was used to ensure the accuracy of end point determination. Rate constants for Phase II, conversion of 2,4-dinitrophenyl phosphate dianion to inorganic phosphate, were also calculated at pH > 9.4.

The effect of co-surfactants. Predetermined amount of CTAB (0.2 M) was added to the reaction mixture at t=0 and the rate constants were calculated at pH 9.4 ± 0.1 for many diester phosphates. A blank kinetic run for reaction mixture containing CTAB and diester phosphate was carried out for every condition investigated.

With slow reactions, addition of CTAB was made after obtaining enough data points for determination of initial rate constant. The rate constant for the reaction in the presence of CTAB (below and above its CMC at pH 9.4) was calculated by monitoring the reaction over 5 to 7 days.

N-Brominating Agents

Stability determination of N-bromo compounds at various buffers

General procedure A. N-Brominating agent (0.01-0.02 g) was accurately weighed and dissolved in 5 mL buffer. The solutions each were diluted to 50 mL with the corresponding buffer and stored in the dark at room temperature. Five millileter aliquots were taken at predetermined time intervals after storage and analyzed for positive bromine (Br^+) concentration by iodometric titration using standardized sodium thiosulfate relative to a blank sample. The half lives of stabilities of the N-bromo compounds tested by this method were calculated from the plot of % bromine against time.

General procedure B. A stock solution of N-brominating agent ($\approx 5 \times 10^{-2}$ M) was prepared in deionized water or buffer. The solution was stored in an amber bottle at room temperature. A 200 μL of sample was withdrawn at predetermined time intervals and diluted with 2.5 mL buffer solution in a 1 cm thick UV cell and equilibrated at 35°C for 5 to 10 min. The change in the concentration in N-brominating agent (Br^+) was determined either by monitoring the absorbance of the compound or hypobromite ion (OBr^-, 334 nm) relative to a sample at time zero in the corresponding buffer. The results are plotted as percentage concentration against time to give in general a semi-logarithmic plot, and the half lives were determined from the plot.

Iodometric titration[51]

The indirect iodine method was applied to determine the active bromine in aqueous solutions. The method was based on the following equations:

$$RN\text{-}Br + H^+ + 2I^- \rightarrow RN\text{-}H + Br^- + I_2$$

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^-$$

The iodide is oxidized to the free iodine by the formation of hypobromite ion from the N-brominating agent in the aqueous solution. The free iodine is then titrated with standard thiosulfate solution (0.02 M) in order to determine the active bromine (OBr^-) in solution. Two moles of thiosulfate are consumed for every mole of active bromine in the indirect iodometric titration.

Reagents and solutions prepared are: (1) sodium thiosulfate solution (1.00 ± 0.05 x 10^{-2} M) from anhydrous $Na_2S_2O_3$ ("Gold Label":Aldrich) (b) potassium iodide (iodate free, "Gold label" (99.99%):Aldrich) (c) starch-iodide paper (Fisher) (d) glacial acetic acid (e) standard potassium iodate solution (0.3075 g dry KIO_3 dissolved in deionized water to a total volume of 100 mL).

General method.[51] A volume of 5 to 10 mL of stock solution (10^{-2} M) of N-brominating agent (or accurately weighed ≈0.03 g of solid in 10 mL deionized water) was transferred to a scintillation vial or Erlenmeyer flask and about 10 mL deionized water was added, followed by 0.2 g of iodate free potassium iodide and 1 mL of glacial acetic acid. The solution was kept at 35°C for 10 min and the librated iodide was titrated with standardized (10^{-2} M) $Na_2S_2O_3$ solution until the starch iodide paper turned from violet to colorless. Bromine content was determined as an average of two determinations. In case, where the aqueous insolubility of N-bromo compound was experienced, the compound was dissolved in minimum amount of DMF and diluted with water. The error in the determination of bromine content is ± 3%.

Standardization of sodium thiosulfate.[51] The sodium thiosulfate solution was standardized by titrating aliquots of potassium iodate solution. Aliquots of potassium iodate were added to a 125 mL Erlenmeyer flask. A 10 mL portion of 15% KI was added to the flask. This was followed by 10 mL of 6M H_2SO_4. Free iodine was liberated in the solution according to the following equation:

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3H_2O + 3I_2$$

This solution was then titrated with $Na_2S_2O_3$ solution until the violet color of starch-iodide paper almost disappeared. The thiosulfate solution standardization was checked several times during the course of experiments described above to make sure that it had not deteriorated.

Determination of equilibrium constant between N-bromosuccinamide (NBS) and N-brominating agent.[45]

The equilibrium constant K_{equ} was determined spectrophotometrically at a wavelength (307 nm) where NBS has no appreciable absorbance and unhindered by the absorbance spectrum of free oxazolidinone. The equilibrium constant was calculated using the equation described in Chapter II. All initial concentrations of N-brominating agents were kept below 10^{-3} M. The positive bromine content of the stock solutions were predetermined iodometrically. Standard calibration curves of absorbances of NBS, succinimide and N-brominating agents were determined at various wavelengths including the wavelengths of K_{equ} determination.

The reaction was started by addition of succinimide (10^{-3} M) to N-brominating agent (≈ equal molar) in 2.5 mL buffer solution in a 3 mL screwcapped UV cell (1 cm path length). The cell was equilibrated in the cell compartment at 25°C, and the absorbance changes were monitored at wavelengths of interest until the equilibrium (no appreciable changes in absorbance spectrum) was reached. Each K_{equ} value that is listed in Table 2-16 is an average of four trials within 0.03 difference.

Estimation of release of active bromine (OBr^-) in solutions of N-brominating agents at various buffer.

Aliquots of stock solution of N-brominating agent were added to 2.5 mL buffer in a UV cell to make up a concentration around 10^{-3} M. The optical density was recorded at $_{max}$ for N-bromo compounds and $_{max}$ for hypobromite ion species (338 nm) at constant temperature. The percentage release of active bromine (OBr^-) from the compound was measured as the percentage of the absorbance values. The absorbance of equivalent concentrations of N-bromo oxazolidinone and NaOBr at alkaline pH are similar.[45]

Kinetic studies of Paraoxon hydrolysis by N-brominating agents

The kinetic runs were conducted according to the method (Method 1) described for hydrolysis of phosphate esters by ECNR. All reactions were carried out in buffer systems of ionic strength 0.8. The concentration of N-brominating agent was kept at 10^{-3}M, 15 to 20 fold more than the concentration of Paraoxon (10^{-5} M) in order to achieve pseudo first order condition.

The reaction was started by adding 2 to 5 μL of a 1% v/v solution of Paraoxon in dioxane to a predetermined concentration of N-brominating agent in 2.5 mL buffer at the temperature investigated. Due to the decomposition tendency of the N-brominating agent in the buffer solution equilibration time prior to addition of Paraoxon was limited to 5 minutes.

The reaction was followed for a minimum of 3 half lives (or 4h) and rate constants were calculated from half lives obtained from semilogarithmic correlation of $A_\infty - A_t$ against time. All stock solutions of N-brominating agents were checked for their active bromine content prior to use and values were corrected accordingly.

Reactivity studies of N-brominating agents with chloroethyl ethylsulfide (mustard agent model)

General procedure (G.C. method). A t=0, the stock solution (10^{-2}M) of N-brominating agent (400 μL) was added to 40 mL buffer containing 40 μL of stock solution (10^{-1} M) of chloroethyl ethylsulfide in cyclohexane and stirred at room temperature. At 60 sec intervals, 5 mL aliquots were withdrawn, shaken with 1 mL cyclohexane in a scintillation vial and mustard agent was extracted into the cyclohexane layer. An excess of sodium chloride was added to the scintillation vial to improve extraction efficiency. Using a micropipet 100 μL of the organic phase was transferred into a sealed ampule for GC assay. Diethyl sulfide and diisopropyl sulfides were used as GC standards for mustard agent. N-brominating agent = 1.0×10^{-3} M mustard = 1.0×10^{-3} M

GC assay for mustard agent.

Column : chromosorb 101 80/100, 6'x 1/8'
N_1 flow : 60 mL/min
Temp : Column : 130°C
    Injector port : 160°C
    Detector : 200°C (FID)
Sample size : 2 μL

Mustard agent retention time : ~11 min
Cyclohexane retention time : ~5 min

Formulation of tetrabromo dimethylglycouril (54(b)) with hydroxypropyl β-cyclodextrin (HPCD) solution

A 50% w/v (or 33%) HPCD (obtained from Pharmatec, Inc.) was added to tetrabromo dimethyl glycouril (0.954 g) in a minimum amount and sonicated until complete solution resulted. The insoluble micro particles were removed by filtration through a sintered glass funnel and the filtrate was either stored in an amber bottle or freeze-dried and stored in the dark. The inclusion complex was assayed for active bromine content iodometrically at predetermined time intervals and the values were compared with the initial content.

The HPCD-tetrabromide inclusion complex was prepared with buffered (borate, pH 8.3, 0.2 M) HPCD solution using essentially the method described above and the active bromine content was compared with that of unbuffered HPCD complex.

Selective oxidation of 7α-hydroxy group in cholic acid to give 3α, 12α-dihydroxy-7-ketocholanic acid by NBMOS (47)

Cholic acid (Sigma, 0.43 g, 1 mmol) was dissolved in a solution of sodium bicarbonate (0.25 g, 3 mmol) in 8 mL of water. This solution was treated with a solution of NBMOS (1.25 equivalents) in 5 mL water at room temperature in a analogus manner to that described for NBS[40] and 3-bromo-4,4-dimethyl-2-oxazolidinone.[45] The resulting precipitate after acidification was separated and the unreacted cholic acid was removed as a precipitate with ethyl acetate. The filtrate was evaporated and once again precipitated with ethyl acetate and the final filtrate was dried to give the oxidized product (33% yield). The product obtained was found to be identical in m.p. and TLC to an authentic sample of 3α, 12α-dihydroxy-7-ketocholanic acid which had been prepared by oxidation of cholic acid using NBS and NBDMO (47): TLC (acetone/1% acetic acid): R_f=0.81; m.p./106-110°C (lit.[40,45] 111-114 for crude product). IR (KBr): 3400, 2960, 1700, 1420, 1250 and 1050 cm^{-1}.

^{1}H NMR spectrum of the product was found not to match that of cholic acid. Mass spectrum (EI) of product showed m/e 406 (M^+), 407 (M^+ +1) and mass spectrum (EI) of cholic acid showed m/e 408 (M^+), 409 (M^+ +1).

CHAPTER IV
SUMMARY AND CONCLUSIONS

The excessively charged nucleophilic reagents (ECNR) containing hydroxamate and oximate nucleophilic heads as well as models of "aged" organophosphate inhibited acetylcholinesterase were designed, synthesized and characterized.

The most obvious synthetic route to the preparation of (hydroxyimino)-1-alkoxycarbonymethylpyridinium salts, the esterification of the corresponding pyridinium-1-acetic acid, was found unsuitable. A relatively difficult approach, through the reaction of di (or mono) quaternized bromoester with (hydroxyimino) pyridine in the final step gave target oxime ECNRs in moderate yield. New approaches and routes were used in the synthesis of hydroxamic acid ECNR. A well known method described by Kunitake et al.[18] for the preparation of N-substituted hydroxamic acid gave no desired product in this case. Instead an approach beginning from O-protected hydroxylamine which in three direct steps gave N-ethyldiethylamine substituted hydroxamate was used. The hydroxamate was then transformed to the desired product 14 in a three steps. The route chosen for the synthesis of hydroxamic acid ECNR involved deprotection of the protecting group and hydrogenation of the double bond all in one final step instead of three additional steps that is usually required otherwise. Overall, the synthetic scheme provided pure target molecules without a need for purification through the ion-exchange or other chromatographic means despite the low yield. Possibly, use of 3-(4-imidazolyl)propionic acid instead of 3-(4-imidazolyl)propenionic acid and short reaction time would increase the final yield by reducing the side reaction formation of Micheal adduct.

Organophosphate diesters were synthesized conveniently through a new procedure, avoiding dealkylation of triesters, by a modified method used in nucleotide synthesis. The direct alkoxylation of 4-nitrophenyl phosphodichloridate and subsequent hydrolysis of the intermediate provided the desired form of the diester. Some new organophosphate triesters were synthesized using a method described by Loew and Lacey[26].

Some novel, stable, water soluble, low bromine potential N-bromo 2-oxazolidinone systems were prepared using 4-hydroxy-4-methyl-2-oxazolidinone as the major synthon. The use of a relatively uncommon brominating agent, t-butyl hypobromite, provided sodium bromide free N-bromo compounds. A versatile precursor to water soluble and surface active N-bromo systems, 4-(β-diethylamino)ethyloxymethyl-4-methyl-2-oxazolidinone, was synthesized. Attempts at mono substitution of dimethyl glycouril with solubilizing groups were not fruitful. The major problem in the reactions is the insolubility of dimethyl glycouril in all reasonable solvent systems.

All of the cationic nucleophiles (oxime and hydroxamic acid ECNRs) showed marked reactivity against organophosphate triesters, such as Paraoxon and octyl methyl 4-nitrophenyl phosphate. In particular the hydroxamic acid 14 showed acceleration of hydrolysis of octyl methyl 4-nitrophenyl phosphate over 2,000 fold with a second order rate constant of 315.95 M^{-1} min^{-1} at mild basic (pH 9.5) conditions.

The hydroxamic acid ECNR (14) showed exceptional rate of enhancement over 1,700 fold in the hydrolytic cleavage ($t_{1/2}$=150 minutes) of phenyl 4-nitrophenylphosphonate anion at pH 9.5. This type of rate acceleration has not been reported for any known nucleophilic or catalytic system against phosphorus ester monoanions. Compound 14 showed pronounced hydrolysis of bis 2,4-dinitrophenyl phosphate over 1 to 3 minutes (4,000 fold acceleration), however, bis 4-nitrophenyl phosphate showed relatively low reactivity with 14. Unexpectedly low reactivity of dodecyl, octyl and other long chain 4-nitrophenyl phosphates suggests that the hydrophobic phosphate diester anions are incorporated deep inside the micelle far from the reach of the nucleophilic head of the ECNR. The oxime ECNRs showed relatively little reactivity with the phosphorus ester monoanions.

The kinetic evidence suggests that the reactions of these ECNRs with phosphate diester and phosphonate monoanions are mostly governed by the electrostatic interactions between the anionic substrate and cationic nucleophile rather than the hydrophobic solubilization in the micelle. It appears from the molecular modelling that the reaction of 14 with phosphate monoanions depends largely on the effective interactions of the negatively charged phosphoryl group with the positively charged tertralkylammonium moiety of the reagent. The kinetic process seems to be second order with respect to the concentration of phosporus substrate and reagent.

In conclusion, the overall reactions of ECNR with a phosphate monoanion (or phosphonate) tend to support the hypothesized mode of action: (a) neutralization of repulsive charge and (b) simultaneous attack on the phosphorus atom. Some selected ECNRs from the groups described or direct analogs of these reagents will be tested for in vivo and in vitro reactivation of poisoned aged AChE.

The N-bromo compounds (3-bromo-4-methyl-2-oxazolidinone-4-yl)methyl sulfate (47) and 3-bromo-4-succinoyloxymethyl-4-methyl-2-oxazolidinone (49) showed high stability and water solubility while still maintaining the apparent necessary structural characteristic of a 5-membered ring. Among these the sulfate (47) showed increased stability in buffered solutions and high reactivity with Paraoxon relative to the hemisuccinoyl analogue (49). Compound 47 also caused a rapid hydrolytic cleavage of the mustard model, and appears to have selective reactivity similar to the widely used N-bromosuccinimide in other reactions.

In conclusion, the low bromine potential compounds 47 and 49 exhibit the necessary characteristics as stable and water soluble sources for positive bromine. However, the self oxidizing tendency of the succinoyl analogue (49) in basic solution sets a limit to its use as decontaminating agent. Despite the insolubility, tetrabromo dimethyl glycouril was found to be an exceptionally stable source capable of providing a high yield of active bromine per molecule. Some selective manipulation of the structural frame to include distal polar groups through a synthetic strategy that involves condensation of unsymmetrical (N-substituted urea) or symmetrical (urea) starting materials may yield a more promising universal decontaminant.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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